Remarks/Arguments

Claims 45-63, 65-74, 76-114 remain in the application. Claims 64, 75, and 88 - 111 have been cancelled and claims 45, 46, 47, 49, 54, 55, 56, 58, 60, 68, 69, 70, 71, 80, 81, 82, 87, 112, and 113 have been amended. Enabling support for the amendments can be found in the application as filed and in the previously pending claims, and therefore, no new matter or new issues are contained in the amendments. Reconsideration of the present application and allowance of the resulting Claims is respectfully requested in view of the amendments and following remarks.

I. Rejection Under 35 U.S.C. §112 First Paragraph

A. Claims 45-51, 53-87, and 112-114

Claims 45-51, 53-87, and 112-114 were rejected under 35 U.S.C. §112, first paragraph. In particular, the Office Action contends that the specification does not specifically define any of the gene sequences that fall within the genus nor does the specification define any structural features commonly possessed by members of the genus that distinguishes them from others. Applicants respectfully traverse this rejection.

Rubredoxin and β-amyloid are widely known by those skilled in the art, especially in regards to structure/form, source, and function. Applicants reiterate that the present invention is directed to a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin and β-amyloid fusion protein. The exact origin of the rubredoxin and β-amyloid are not critical to the nature of the invention. Applicants, in the previous response to Office Action, have supplied numerous publications detailing the source and function of rubredoxin and β-amyloid peptide.

In response, the Examiner cited to the *Eli Lilly* (43 USPQ2d 1398) case which does not apply to the present invention. In the *Eli Lilly* case, all mammalian forms of insulin were claimed by the Applicants but only the sequence of **one mammal was provided** at the time of the invention and no other sequences were known in the art. The present invention is not related to an area of art in which there is only one known protein or peptide sequence for rubredoxin or

β-amyloid. One skilled in the art could construct numerous rubredoxin and β-amyloid fusion proteins based on the specification and the knowledge available in the art.

Further to the publications previously presented to the Examiner by the Applicants, which supports the numerous examples of known sequences of rubredoxin and β-amyloid peptides, Applicants enclose additional references which clearly support the fact that rubredoxin and β-amyloid are widely known in the art in regards to structure and function.

The Office Action suggests that the structure of rubredoxin is not supported in the specification; however, the structure of rubredoxin was widely known in the art at the time of filing. In Appendix A, Applicants enclose numerous sources to ascertain the structure for rubredoxin available at the time of filing the present application. The information supplied was obtained from www.pubmed.com. One skilled in the art could easily obtain 3-D structures of rubredoxin from *Pyrococcus furiosus, Desulfovibrio gigas, Clostridium pasteurianum, Desulfovibrio desulfuricans,* and *Delsulfovibrio vulgaris* as seen in Appendix A. All of these 3-D structures were available well before the time of filing of this application, dating back to 1984 by K.D. Watenpaugh *et al.*

In Appendix B, Applicants enclose various sources to obtain the protein sequences for rubredoxin available at the time of filing the present application. Appendix B provides the sources and the sequences for rubredoxin which one of ordinary skilled in the art could obtain. The sources for the sequences of rubredoxin include *Azotobacter vinelandii*, by Menon *et al.* 1994; *Clostridium pasteurianum*, by Mathieu *et al.*, 1993; *Desulfovibrio vulgaris*, by Dolla A. *et al.* 1993; *Stenotrophomonas maltophilia*, by Lee N.R. *et al.* 1996; *Pyrococcus furiosus*, by Blake P.R., *et al.* 1993; *Heliobacillus mobilis*, by Lee W.Y. *et al.* 1995; *Desulfovibrio baarsii,* by Pianzzola M.J., *et al.*, 1996; *Clostridium diolis*, by Gerard, P. Mar. 1997; *Clostridium acetobutylicum*, by Belouski, E. Mar. 1997; *Desulfovibrio gigas*, by Brushi, M., Sep. 1998; and *Desulfovibriode desulfuricans,* by Sieker, L.C. *et al.,* Oct. 1998. It is apparent from this non-exhaustive list that the sequence of rubredoxin and its various sources were widely known.

In Appendix C, Applicants enclose references from the database to ascertain the structures for β-amyloid peptide available at the time of filing the present application. Various structures of Beta-amyloid peptide were made available by J. Talaous *et al.* in 1994, M. Coles et

al. in April 1998, A.A. Watson et al. in April and June 1998 taken from studies of Alzheimer's disease, methionine oxidized, and NMR studies.

In Appendix D, Applicants enclose the various sources for the sequences of β-amyloid available at the time of filing the present application. The sources for the sequence of β-amyloid include *Mus musculus,* by Yamada, T. *et al.* 1993 and *Homo Sapiens*, by Zain, S.B. 1994. Other sequences of β-amyloid peptide are widely available in U.S. Patents Nos. 5,434,050 (filed on 1995), 5,441,870 (filed on 1995), 5,525,714 (filed on 1996), 5,552,426 (filed on 1996); and 5,605,811 (filed on 1997). All of these β-amyloid pepetide sequences were published well before the filing of Applicants' application.

In Appendix E, Applicants resubmit the articles and publications supporting the widely known information relating to rubredoxin and β-amyloid available at the time of filing the present application, which are summarized in the table below.

Crystallographic study of rubredoxin from the bacterium Desulfovibrio desulfuricans strain 27774. Sieker LC, Jensen LH, Prickril BC, LeGall J *J Mol Biol* 1983 Nov 25 **171:1** 101-3
Purification and properties of ferredoxin and rubredoxin from Butyribacterium methylotrophicum. Saeki K, Jain MK, Shen GJ, Prince RC, Zeikus JG *J Bacteriol* 1989 Sep **171:9** 4736-41
Expression of a synthetic gene coding for the amino acid sequence of Clostridium pasteurianum rubredoxin. Eidsness MK, O_Dell SE, Kurtz DM, Robson RL, Scott RA *Protein Eng* 1992 Jun **5:4** 367-71
Synthesis and characterization of Desulfovibrio gigas rubredoxin and rubredoxin fragments. Christensen HE, Hammerstad_Pedersen JM, Holm A, Iversen G, Jensen MH, Ulstrup J *Eur J Biochem* 1994 Aug 15 **224:1** 97-101
Isolation, characterization, and primary structure of rubredoxin from the photosynthetic bacterium, Heliobacillus mobilis. Lee WY, Brune DC, LoBrutto R, Blankenship RE *Arch Biochem Biophys* 1995 Apr 1 **318:1** 80-8
Recombinant two-iron rubredoxin of Pseudomonas oleovorans: overexpression, purification and characterization by optical, CD and 113Cd NMR spectroscopies. Lee HJ, Lian LY, Scrutton NS *Biochem J* 1997 Nov 15 **328 (Pt 1):** 131-6
A Beta40 Is A Major Form Of Beta-Amyloid In Nonhuman Primates. Gearing M, Tigges J, Mori H, Mirra SS Neurobiol Aging 1996 Nov-Dec 17:903-8

Amyloid Beta Protein In Rat Soleus Muscle In Chloroquine-Induced Myopathy Using End-Specific Antibodies For A Beta 40 And A Beta 42: Immunohistochemical Evidence For Amyloid Beta Protein. Tsuzuki K, Fukatsu R, Takamaru Y, Yoshida T, Hayashi Y, Yamaguchi H, Fujii N, Takahata N Neurosci Lett 1995 Dec 202:77-80
Homology Of The Amyloid Beta Protein Precursor In Monkey And Human Supports A Primate Model For Beta Amyloidosis In Alzheimer's Disease. Podlisny MB, Tolan DR, Selkoe DJ Am J Pathol 1991 Jun 138:1423-35
A Comprehensive Study Of The Spatiotemporal Pattern Of Beta-Amyloid Precursor Protein Mrna And Protein In The Rat Brain: Lack Of Modulation By Exogenously Applied Nerve Growth Factor. Neve RL, Valletta JS, Li Y, Ventosa-Michelman M, Holtzman DM, Mobley WC Brain Res Mol Brain Res 1996 Jul 39:185-97
Permeability And Residual Plasma Volume Of Human, Dutch Variant, And Rat Amyloid Beta-Protein 1-40 At The Blood-Brain Barrier. Poduslo JF, Curran GL, Haggard JJ, Biere AL, Selkoe DJ Neurobiol Dis 1997 4:27-34
Zinc-Induced Aggregation Of Human And Rat Beta-Amyloid Peptides In Vitro. Esler WP, Stimson ER, Jennings JM, Ghilardi JR, Mantyh PW, Maggio JE J Neurochem 1996 Feb 66:723-32

As seen from these sources, Applicants should not be required to disclose each and every single sequences, structure, or function in the application as they are widely known by those skilled in the art.

As emphasized, Applicants' invention as currently claimed is related to the construct of the rubredoxin in combination with the β-amyloid peptide. Because both the genus and many examples of specific species within the genus are widely known in the art for each of rubredoxin and β-amyloid peptide, all that is required is that the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the presently claimed subject matter. *See In re Kaslow,* 707 F.2d 1366, 1375, 217 U.S.P.Q. 1089, 1096 (Fed. Cir. 1983).

Applicants reemphasis that the *Eli Lilly* case is fact specific and does not apply to the present invention, because rubredoxin and β-amyloid peptide are well known in the art in regards to form/structure, source, and function. Thus, as stated by the Federal Circuit "an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains." *In re Hayes Microcomputer Products Inc. Patent Litigation*, 982 F2d. 1527, 1534-35 (Fed. Cir. 1992). The prior art provides numerous

organisms which encode for rubredoxin from a wide variety of bacteria. At the time of the claimed invention, Applicants have stated that "rubredoxin from numerous different organism have been isolated, and the amino acid sequences of various rubredoxins and the genes encoding various rubredoxins have been published." (Specification page 25, lines 27-29). In the present application, Applicants disclose the use of *D. vulagaris* as a source for a gene that encodes rubredoxin as a representative example, which is conventional in the art, and known to one of ordinary skill in the art. The selection of the source for encoding rubredoxin is sufficiently developed so as to put one of skill in the art in possession of the steps of the method and the elements of the composition. In other words, one skilled in the relevant art would understand what is intended by the claimed invention and how to carry it out. In light of the evidence submitted, one of ordinary skill in the art would understand the invention to be related to fusion protein comprising a rubredoxin fusion partner, fused directly or indirectly to a ß-amyloid peptide, together with methods and materials for producing the fusion protein in a host cell and purifying the fusion protein, and not critically dependent upon the origin of the rubredoxin or β-amyloid in each case. The courts have pointed out that "[n]ot every last detail [of an invention need] be described [in a patent specification], else patent specifications would turn into production specifications, where there were never intended to be." *In re Gay,* 309 F.2d 769, 774, 135 U.S.P.Q. 311, 316 (C.C.P.A. 1962).

Further, the claimed invention is not directed to a particular polynucleotide sequence; rather, the claims are directed to a construct describing a particular way of combining types of sequences (e.g., a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β-amyloid peptide). The claims are therefore directed to a novel combination of known types of sequences, and are not drawn to a particular sequence. As such, Applicants are not required to disclose every possible sequence that can be used interchangeably in the claimed polynucleotide constructs. The novelty of the invention does not lie in the particular sequence used, but lies in the structure and combination of the construct. As least for the above mentioned remarks, Applicants respectfully request withdrawal of this rejection.

B. Claim 52

Claim 52 remains rejected under 35 U.S.C. §112, first paragraph, by the Examiner as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention." Applicants respectfully traverse this rejection.

The Examiner states that only a "schematic of the vector rRUBEX23 is described and only a portion of the sequence is disclosed...therefore, it would involve an undue experimentation to make this vector." Applicants reiterate that claim 52 provides sufficient description that would not require undue experimentation to those of ordinary skilled in the art. Applicants reiterate that the specification clearly defines pRUBEX3, which comprises a histidine tag and a Factor Xa cleavage site of pRUBEX2 as well as containing "as part of the intervening spacer a portion of the multiple cloning region to facilitate cloning of the nucleotide sequence encoding the fused polypeptide into the vector". pRUBEX2 and pRUBREX1 are in turn derived from the "coding sequence for *D. vulgaris* rubredoxin and the fused polypeptide are directly linked" (Specification, page 19, line 31 to page 20, line 9). As stated in the previous section, rubredoxin is well known in the art in regards to form, function, structure and source. Thus, one of ordinary skilled in the art could easily ascertain the coding sequence for coding sequence for *D. vulgaris* rubredoxin. Because the Examiner failed to consider the publications previously sent in support of these facts, Applicants submit and refer the Examiner to Appendix A, B, and D to represent that the coding sequence for rubredoxin which could be easily obtained from readily available source. Thus pRUBEX3 could be ascertained with the teachings of the specification because the source, sequence, and structure is widely known at the time of the present invention.

Applicants have also provided, in Figure 1, a schematic of the vector pRUBEX3, including the Multiple Cloning Region (MCR) and the nucleotide sequence of a portion of pRUBEX3 together with the amino acid sequence encoded. Not only is pRUBEX3 defined and exemplified in Figure 1, Applicants have also provided in Example 1, the method to yield pRUBEX3 (Specification, Example 1, page 25, line 23 to page 28, line 24; **with emphasis** on page 28, lines 23-24). Thus, the specification has disclosed in a manner that one skilled in the art would be able to practice the invention without an undue amount of experimentation. *See In re Colianni,* 561 F.2d at 224, 195 U.S.P.Q. at 153*;see also M.P.E.P* §2164.02.

The Examiner has also relied on 37 C.F.R. §1.802, as a basis for this rejection, which states:

> (a) Where an invention is, or relies on, a biological material, the disclosure **may include** reference to a deposit of such biological material.
>
> (b) Biological material need not be deposited unless access to such material is necessary for the satisfaction of the statutory requirements for patentability under 35 U.S.C. 112. **Biological material need not be deposited**, *inter alia*, if it is known and readily available to the public or **can be made or isolated without undue experimentation**.

(emphasis added; irrelevant sections omitted).

As stated above, Applicants are not required to submit the biological material if it can be made or isolated **without undue experimentation**. Applicants have articulated above that pRUBEX3 has been defined in the specification and exemplified in Figure 1 and Example 1. Because pRUBEX3 would not require an undue amount of experimentation to practice to those skilled in the art, Applicants are not required to deposit this biological material as it has been satisfied under the statutory requirements of 37 C.F.R. §1.802 and 35 U.S.C. §112. *See also Amgen, Inc, v, Chugai Pharm.* Co., 927 F.2d at 1210, 18 U.S.P.Q.2d at 1024 (inventor did not need to deposit cells used in the invention's best mode if one skilled in the art could reproduce without undue experimentation); *see also Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1385, 231 U.S.P.Q. at 94 (The mere fact that the best mode requires sophisticated competent people to engage in a labor-intensive and time-consuming process does not amount to a best mode violation in the absence of a deposit. The key to the analysis is whether the scientists must engage in *undue experimentation*).

Applicants have clearly enabled pRUBEX3 and have provided sufficient description and evidence to overcome the rejection. Thus, Applicants respectfully request withdrawal of this rejection.

II. Rejection Under 35 U.S.C. §112, Second Paragraph

A. Claim 64

The Office Action rejected claim 64 under 35 U.S.C. §112 which Applicants have cancelled and thus is no longer an issue. Applicants respectfully request withdrawal of this rejection.

B. Claim 112

The Office Action rejected claim 112, under 35 U.S.C. §112, second paragraph for being unclear. Applicants have amended claim 112 based on the Examiner's suggestion for clarity and thus respectfully request withdrawal of this rejection.

III. Rejection Under 35 U.S.C. §103

The Examiner has rejected claims 45-51, 53-63, 65-87, and 112-114 under 35 U.S.C. §103(a) as unpatentable over Ueno *et al.* and Dobeli *et al.* Applicants respectfully traverse this rejection.

Applicants have amended the claims to further highlight the significance of the solubility of the rubredoxin and the preferred protein of interest ß-amyloid fusion protein in a recombinant expression system. Applicants emphasize that the Federal Circuit has held that evidence of a teaching, suggestion, or motivation to combine may flow from references themselves or from the knowledge of one of ordinary skilled in the art, but may **<u>not</u>** flow from the Applicants' disclosure. *See Pro-Mold Tool Co. v. Great Lakes Plastics, Inc.*, 75 F.3d 1568, 1573 (Fed. Cir. 1996), *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). Importantly, this evidence of a teaching, suggestion, or motivation, to combine must be "clear and particular". *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999).

In the present case, the Examiner has not pointed to any actual evidence in the cited references that would have led one of ordinary skill in the art to combine the teachings in a manner suggested by the Examiner. As stated by the Federal Circuit, this evidence must be clear and particular. Broad conclusory statements regarding the teachings of multiple references, standing alone, are not "evidence". *Id.* In the outstanding Office Action, the Examiner has used the teachings of the present invention to show that it would have been obvious for Ueno *et al.* to

combine rubredoxin with the β-amyloid taught by Dobeli *et al.* Neither references, alone or in combination, suggests a soluble rubredoxin-B-amyloid combination as presently claimed.

Applicants emphasize the unexpected benefits of the present invention with respect to the particular rubredoxin and β-amyloid fusion construct now claimed. The Dobeli *et al.* 13.8 kD fusion with the amyloid is not soluble and goes into the inclusion bodies. Further, nowhere in the reference does it teach or suggest an iron containing nucleotide having red color for easy tracking. Not only is there no suggestion or motivation, the Dobeli *et al.* reference teaches away from the presently claimed invention. The Dobeli reference teaches a chemical cleavage of beta-amyloid from fusion which causes side reactions. The Ueno *et al.* reference does not teach or suggest the use of any β-amyloid. Further the Ueno disclosure does not teach rubredoxin constituent that is soluble, nor does it teach a rubredoxin constituent that goes into the inclusion bodies. Thus, even if all the references were to be combined, there would be no teaching or suggestion of the claimed invention. Prior to the present application, no one had described that rubredoxin was an advantageous fusion partner for creating soluble β-amyloid expression products that are also colorimetric.

Applicants highlight the Kohli *et al* reference[1] (previously provided) only to show that the presently claimed invention is novel and not obvious. Five years after the filing of the present application, Kohli *et al* published their findings of surprising utility of rubredoxin expression system as claimed by the present invention. Based, on the foregoing remarks, Applicants respectfully request withdrawal of this rejection.

IV. Conclusion

In summary, Applicants have particularly pointed out and distinctly claimed the subject matter which Applicants regard as the invention, and the subject matter of the present invention is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Furthermore, the subject matter of the present invention is neither disclosed nor rendered obvious by any of the cited documents or by any combination of the documents if the

[1] Kohli, Bernhard *et al.*, *Protein Expression & Purification* 28 (2003) 362-367.

skilled person would have combined them at all. Therefore, Applicants respectfully request withdrawal of the present rejections and allowance of the claims.

The Examiner is encouraged to call the undersigned attorney at 404-853-8081 if doing so will facilitate prosecution of the application. No fees are believed to be due at this time. However, the Commissioner is hereby authorized to charge any additional fees due or credit any overpayment to Deposit Account 19-5029.

In view of the present amendment and response to the Office Action mailed August 24, 2004, Applicant respectfully requests a timely Notice of Allowance be issued in this case.

Respectfully submitted

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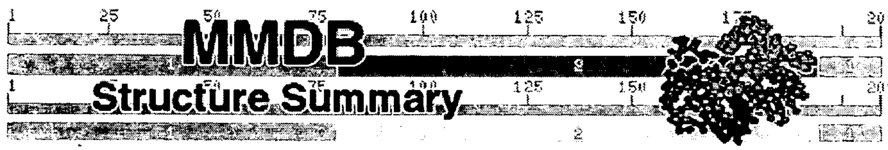
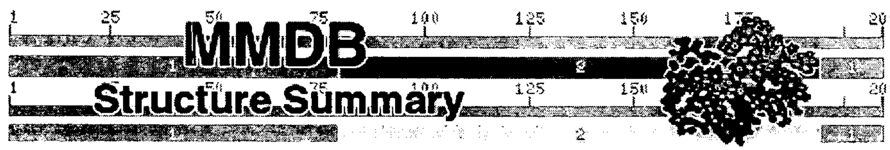


PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin (Oxidized).
Deposition: M.W.Day, B.T.Hsu, L.Joshua-Tor, J.B.Park, Z.H.Zhou, M.W.W.Adams & D.C.Rees, 18-May-92
Taxonomy: Pyrococcus furiosus
Reference: PubMed **MMDB:** 438 **PDB:** 1CAA

View 3D Structure **of** Best Model **with** Cn3D Display NEW **Get Cn3D 4.1!**

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.



PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin (Zn-Substituted) (Nmr, 40 Structures).

Deposition: P.R.Blake, J.B.Park, Z.H.Zhou, D.R.Hare, M.W.W.Adams & M.F.Summers, 10-Jul-92

Taxonomy: Pyrococcus furiosus

Reference: PubMed **MMDB:** 2499 **PDB:** 1ZRP

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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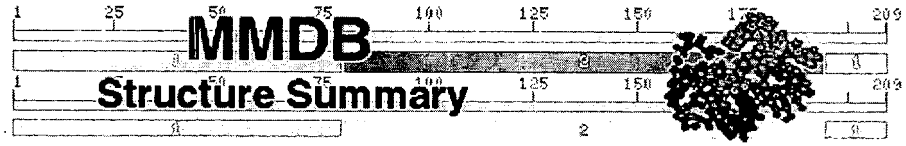


PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin (Reduced).

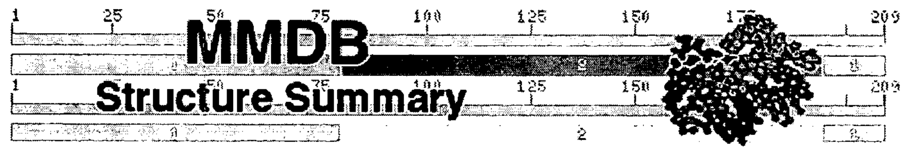
Deposition: M.W.Day, B.T.Hsu, L.Joshua-Tor, J.B.Park, Z.H.Zhou, M.W.W.Adams & D.C.Rees, 18-May-92

Taxonomy: Pyrococcus furiosus

Reference: PubMed **MMDB:** 439 **PDB:** 1CAD

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.



PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin.

Deposition: M.Frey, L.C.Sieker & F.Payan, 17-Mar-88

Taxonomy: Desulfovibrio gigas

Reference: PubMed **MMDB:** 2057 **PDB:** 1RDG

View 3D Structure **of** Best Model **with** Cn3D Display NEW! ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin (Oxidized, Fe(III)) (Unconstrained Model).
Deposition: K.D.Watenpaugh, L.C.Sieker & L.H.Jensen, 15-Oct-84
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 3211 **PDB:** 4RXN

View 3D Structure **of** Best Model **with** Cn3D Display NEW! ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin (Oxidized, Fe(III)) (Constrained Model).
Deposition: K.D.Watenpaugh, 15-Oct-84
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 3292 **PDB:** 5RXN

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

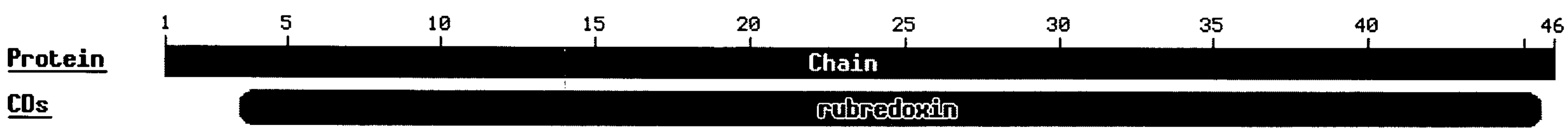
Description: Rubredoxin.

Deposition: R.E.Stenkamp, L.C.Sieker & L.H.Jensen, 16-Jan-90

Taxonomy: Desulfovibrio desulfuricans

Reference: PubMed **MMDB:** 3344 **PDB:** 6RXN

View 3D Structure **of** Best Model **with** Cn3D Display ***Get Cn3D 4.1!***



Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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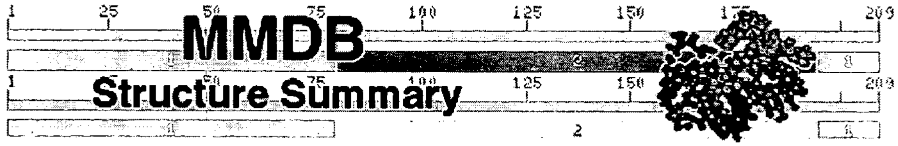
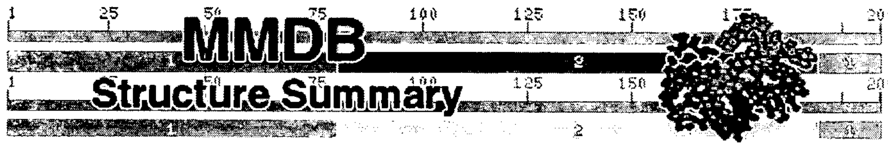


PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin.
Deposition: E.T.Adman, L.C.Sieker & L.H.Jensen, 11-May-90
Taxonomy: Desulfovibrio vulgaris
Reference: PubMed **MMDB:** 3382 **PDB:** 7RXN

View 3D Structure **of** Best Model **with** Cn3D Display NEW! ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.



PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin.
Deposition: Z.Dauter, L.Sieker & K.Wilson, 26-Aug-91
Taxonomy: Desulfovibrio vulgaris
Reference: PubMed **MMDB:** 3418 **PDB:** 8RXN

View 3D Structure **of** Best Model **with** Cn3D Display NEW **Get Cn3D 4.1!**

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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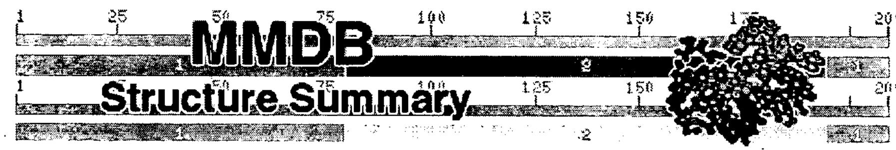

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin (Oxidized, Fe(Iii)) At 1.1 Angstroms Resolution.
Deposition: Z.Dauter, K.S.Wilson, L.C.Sieker, J.M.Moulis & J.Meyer, 13-Dec-95
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 4287 **PDB:** 1IRO

View 3D Structure **of** Best Model **with** Cn3D Display NEW! ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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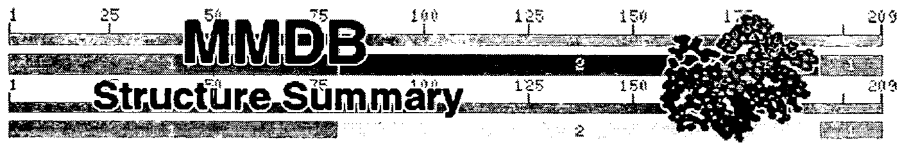

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin (Zn-Substituted) At 1.2 Angstroms Resolution.
Deposition: Z.Dauter, K.S.Wilson, L.C.Sieker, J.M.Moulis & J.Meyer, 13-Dec-95
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 4288 **PDB:** 1IRN

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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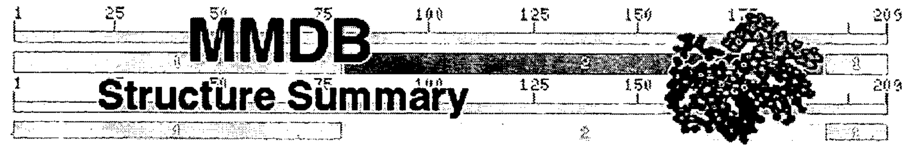

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin (Wild Type) From Pyrococcus Furiosus.

Deposition: R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 24-Aug-98

Taxonomy: Pyrococcus furiosus

Reference: PubMed **MMDB:** 8262 **PDB:** 1BRF

View 3D Structure **of** Best Model **with** Cn3D Display NEW **_Get Cn3D 4.1!_**

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Clostridium Pasteurianum Rubredoxin C42s Mutant.

Deposition: M.Maher, J.M.Guss, M.Wilce & A.G.Wedd, 20-May-98

Taxonomy: Clostridium pasteurianum

Reference: PubMed **MMDB:** 8416 **PDB:** 1BE7

View 3D Structure **of** Best Model **with** Cn3D Display NEW **Get Cn3D 4.1!**

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin From Desulfovibrio Vulgaris Refined Anisotropically At 0.92 Angstroms Resolution.

Deposition: Z.Dauter, S.Butterworth, L.C.Sieker, G.Sheldrick & K.S.Wilson, 21-Dec-97

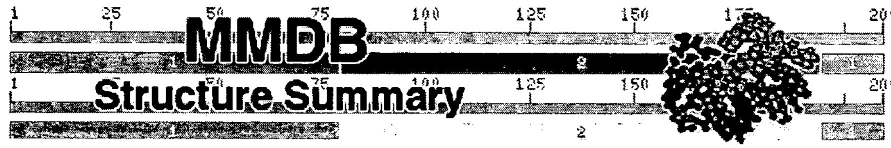
Taxonomy: Desulfovibrio vulgaris

Reference: PubMed **MMDB:** 9637 **PDB:** 1RB9

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

NCBI



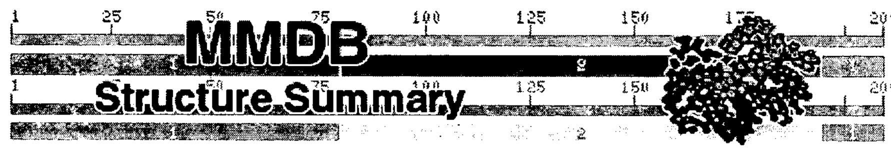
PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Clostridium Pasteurianum Rubredoxin G10a Mutant.
Deposition: M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 26-Nov-98
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 10186 **PDB:** 1B13

View 3D Structure **of** Best Model **with** Cn3D Display NEW! ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

NCBI



PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Clostridium Pasteurianum Rubredoxin G43a Mutant.
Deposition: M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 27-Nov-98
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 10187 **PDB:** 1B2J

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

MMDB Structure Summary

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

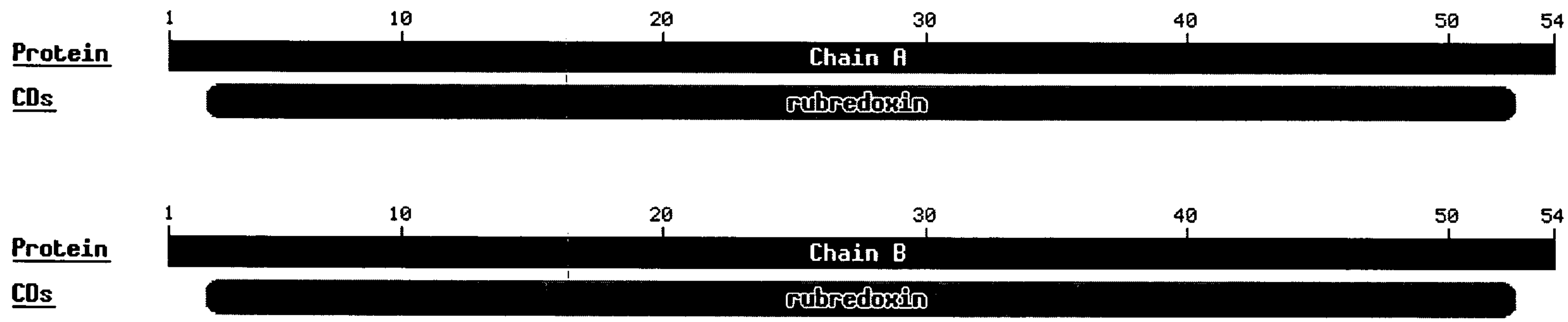
Description: Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.

Deposition: M.J.Maher, J.M.Guss, M.C.J.Wilce & A.G.Wedd, 30-Nov-98

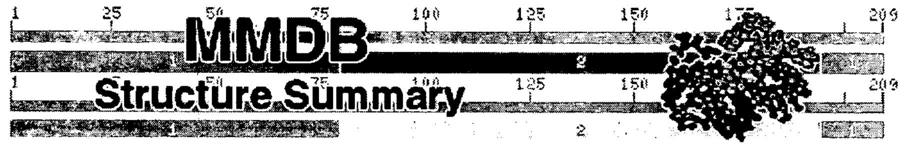
Taxonomy: Clostridium pasteurianum

Reference: PubMed **MMDB:** 10188 **PDB:** 1B2O

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***



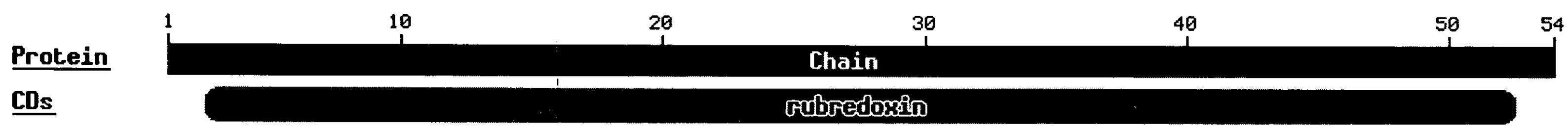
Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.



PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin, Nmr, 20 Structures.
Deposition: I.Bertini, D.M.Kurtz Junior, M.K.Eidsness, G.Liu, C.Luchinat, A.Rosato & R.A.Scott, 23-May-98
Taxonomy: Clostridium pasteurianum
Reference: PubMed **MMDB:** 10206 **PDB:** 1BFY

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***



Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Trigonal Crystal Form.
Deposition: Y.Higuchi & N.Yasuoka, 30-Sep-98
Taxonomy: Desulfovibrio vulgaris
Reference: PubMed **MMDB:** 10323 **PDB:** 1RDV

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Monoclinic Crystal Form.
Deposition: Y.Higuchi & N.Yasuoka, 7-Oct-98
Taxonomy: Desulfovibrio vulgaris
Reference: PubMed **MMDB:** 10357 **PDB:** 2RDV

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

NCBI **MMDB Structure Summary**

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Rubredoxin (Methionine Mutant) From Pyrococcus Furiosus.

Deposition: R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 22-Aug-98

Taxonomy: Pyrococcus furiosus

Reference: PubMed **MMDB:** 11956 **PDB:** 1BQ8

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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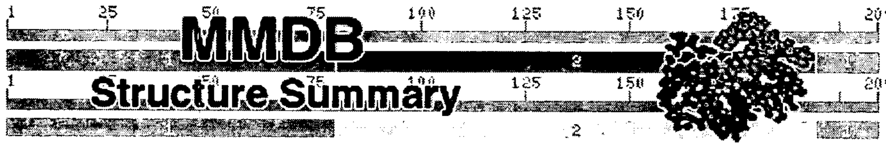

Description: Rubredoxin (Formyl Methionine Mutant) From Pyrococcus Furiosus.

Deposition: R.Bau, D.C.Rees, D.M.Kurtz, R.A.Scott, H.Huang, M.W.W.Adams & M.K.Eidsness, 22-Aug-98

Taxonomy: Pyrococcus furiosus

Reference: PubMed **MMDB:** 11957 **PDB:** 1BQ9

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

1: AAA19505. Reports rubredoxin...[gi:398004]


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LOCUS       AAA19505                  72 aa            linear   BCT 01-JUL-1994
DEFINITION  rubredoxin.
ACCESSION   AAA19505
VERSION     AAA19505.1  GI:398004
DBSOURCE    locus AVIHOXHYP accession L23970.1
KEYWORDS    .
SOURCE      Azotobacter vinelandii
  ORGANISM  Azotobacter vinelandii
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Azotobacter.
REFERENCE   1  (residues 1 to 72)
  AUTHORS   Menon,A.L., Stults,L.W., Robson,R.L. and Mortenson,L.E.
  TITLE     Cloning, sequencing and characterization of the
            [NiFe]hydrogenase-encoding structural genes (hoxK and hoxG) from
            Azotobacter vinelandii
  JOURNAL   Gene 96 (1), 67-74 (1990)
  MEDLINE   91092503
   PUBMED   2265761
REFERENCE   2  (residues 1 to 72)
  AUTHORS   Chen,J.C. and Mortenson,L.E.
  TITLE     Two open reading frames (ORFs) identified near the hydrogenase
            structural genes in Azotobacter vinelandii, the first ORF may
            encode for a polypeptide similar to rubredoxins
  JOURNAL   Biochim. Biophys. Acta 1131 (1), 122-124 (1992)
  MEDLINE   92256484
   PUBMED   1581355
REFERENCE   3  (residues 1 to 72)
  AUTHORS   Chen,J.C. and Mortenson,L.E.
  TITLE     Identification of six open reading frames from a region of the
            Azotobacter vinelandii genome likely involved in dihydrogen
            metabolism
  JOURNAL   Biochim. Biophys. Acta 1131 (2), 199-202 (1992)
  MEDLINE   92305060
   PUBMED   1610901
REFERENCE   4  (residues 1 to 72)
  AUTHORS   Menon,A.L., Mortenson,L.E. and Robson,R.L.
  TITLE     Nucleotide sequences and genetic analysis of hydrogen oxidation
            (hox) genes in Azotobacter vinelandii
  JOURNAL   J. Bacteriol. 174 (14), 4549-4557 (1992)
  MEDLINE   92325046
   PUBMED   1624446
REFERENCE   5  (residues 1 to 72)
  AUTHORS   Garg,R.P., Menon,A.L., Jacobs,K., Robson,R.M. and Robson,R.L.
  TITLE     The hypE gene completes the gene cluster for H2-oxidation in
            Azotobacter vinelandii
  JOURNAL   J. Mol. Biol. 236 (1), 390-396 (1994)
  MEDLINE   94149737
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                     /citation=[4]
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       61 dgdreqfmvv dg
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1: AAA23279. Reports rubredoxin...[gi:144909]


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LOCUS       AAA23279                  54 aa            linear   BCT 26-APR-1993
DEFINITION  rubredoxin.
ACCESSION   AAA23279
VERSION     AAA23279.1  GI:144909
DBSOURCE    locus CLORUB accession M60116.1
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Mathieu,I., Meyer,J. and Moulis,J.M.
  TITLE     Cloning, sequencing and expression in Escherichia coli of the
            rubredoxin gene from Clostridium pasteurianum
  JOURNAL   Biochem. J. 285 (Pt 1), 255-262 (1992)
  MEDLINE   92344580
   PUBMED   1637309
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     Protein         1..54
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                     /note="rubredoxin"
                     /db_xref="CDD:24175"
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                     /note="sequence of product differs from reported protein
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                     /transl_table=11
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Nov 8 2004 13:44:10

1: AAA23380. Reports rubredoxin oxidor...[gi:145115] BLink, Domains, Links

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LOCUS       AAA23380                 126 aa            linear   BCT 26-APR-1993
DEFINITION  rubredoxin oxidoreductase.
ACCESSION   AAA23380
VERSION     AAA23380.1  GI:145115
DBSOURCE    locus DVURBORUB accession M81168.1
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 126)
  AUTHORS   Dolla,A., Fu,R., Brumlik,M.J. and Voordouw,G.
  TITLE     Nucleotide sequence of dcrA, a Desulfovibrio vulgaris Hildenborough
            chemoreceptor gene, and its expression in Escherichia coli
  JOURNAL   J. Bacteriol. 174 (6), 1726-1733 (1992)
  MEDLINE   92193255
   PUBMED   1548224
COMMENT     Method: conceptual translation.
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                     /tissue_lib="NCIMB 8303"
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     Region          2..37
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                     /note="Desulfoferrod_N"
                     /db_xref="CDD:26510"
     Region          41..125
                     /region_name="Desulfoferrodoxin"
                     /note="Desulfoferrodox"
                     /db_xref="CDD:25838"
     CDS             1..126
                     /gene="rbo"
                     /coded_by="M81168.1:2529..2909"
                     /transl_table=11
ORIGIN
        1 mpnqyeiykc ihcgnivevl hagggdlvcc gepmklmkeg tsdgakekhv pviektangy
       61 kvtvgsvahp meekhwiewi elvadgvsyk kflkpgdape aefcikadkv vareycnlhg
      121 hwkaea
//
```

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1: AAA23381. Reports rubredoxin...[gi:145116]


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LOCUS       AAA23381                  52 aa            linear   BCT 26-APR-1993
DEFINITION  rubredoxin.
ACCESSION   AAA23381
VERSION     AAA23381.1  GI:145116
DBSOURCE    locus DVURBORUB accession M81168.1
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Dolla,A., Fu,R., Brumlik,M.J. and Voordouw,G.
  TITLE     Nucleotide sequence of dcrA, a Desulfovibrio vulgaris Hildenborough
            chemoreceptor gene, and its expression in Escherichia coli
  JOURNAL   J. Bacteriol. 174 (6), 1726-1733 (1992)
  MEDLINE   92193255
   PUBMED   1548224
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /strain="Hildenborough"
                     /db_xref="taxon:881"
                     /tissue_lib="NCIMB 8303"
     Protein         1..52
                     /product="rubredoxin"
                     /function="catalyzes the reaction H2 = 2H+ + 2e"
     Region          3..50
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     CDS             1..52
                     /gene="rub"
                     /coded_by="M81168.1:2926..3084"
                     /note="Rubredoxin is a monomer"
                     /transl_table=11
ORIGIN
        1 mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa
//
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1: AAA64797. Reports rubredoxin oxidor...[gi:758676]

BLink, Domains, Links

```
LOCUS       AAA64797                 126 aa            linear   BCT 05-APR-1995
DEFINITION  rubredoxin oxidoreductase.
ACCESSION   AAA64797
VERSION     AAA64797.1  GI:758676
DBSOURCE    locus DVURUBRBO accession M28848.1
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 126)
  AUTHORS   Brumlik,M.J. and Voordouw,G.
  TITLE     Analysis of the transcriptional unit encoding the genes for
            rubredoxin (rub) and a putative rubredoxin oxidoreductase (rbo) in
            Desulfovibrio vulgaris Hildenborough
  JOURNAL   J. Bacteriol. 171 (9), 4996-5004 (1989)
  MEDLINE   89359139
   PUBMED   2549009
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..126
                     /organism="Desulfovibrio vulgaris"
                     /strain="Hildenborough"
                     /db_xref="taxon:881"
     Protein         1..126
                     /product="rubredoxin oxidoreductase"
     Region          2..37
                     /region_name="Desulfoferrodoxin, N-terminal domain"
                     /note="Desulfoferrod_N"
                     /db_xref="CDD:26510"
     Region          41..125
                     /region_name="Desulfoferrodoxin"
                     /note="Desulfoferrodox"
                     /db_xref="CDD:25838"
     CDS             1..126
                     /gene="rbo"
                     /coded_by="M28848.1:199..579"
                     /transl_table=11
ORIGIN
        1 mpnqyeiykc ihcgnivevl hagggdlvcc gepmklmkeg tsdgakekhv pviektangy
       61 kvtvgsvahp meekhwiewi elvadgvsyk kflkpgdape aefcikadkv vareycnlhg
      121 hwkaea
//
```

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1: AAA64798. Reports rubredoxin...[gi:758677]

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LOCUS       AAA64798                  52 aa            linear   BCT 05-APR-1995
DEFINITION  rubredoxin.
ACCESSION   AAA64798
VERSION     AAA64798.1  GI:758677
DBSOURCE    locus DVURUBRBO accession M28848.1
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Brumlik,M.J. and Voordouw,G.
  TITLE     Analysis of the transcriptional unit encoding the genes for
            rubredoxin (rub) and a putative rubredoxin oxidoreductase (rbo) in
            Desulfovibrio vulgaris Hildenborough
  JOURNAL   J. Bacteriol. 171 (9), 4996-5004 (1989)
  MEDLINE   89359139
   PUBMED   2549009
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /strain="Hildenborough"
                     /db_xref="taxon:881"
     Protein         1..52
                     /product="rubredoxin"
     Region          3..50
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     CDS             1..52
                     /gene="rub"
                     /coded_by="M28848.1:596..754"
                     /transl_table=11
ORIGIN
        1 mkkyvctvcg yeydpaegdp dngvkpgtsf ddlpadwvcp vcgapksefe aa
//
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1: AAA97867. Reports rubredoxin reduct...[gi:1276869] BLink, Links

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LOCUS       AAA97867                 264 aa            linear   BCT 25-APR-1996
DEFINITION  rubredoxin reductase.
ACCESSION   AAA97867
VERSION     AAA97867.1  GI:1276869
DBSOURCE    locus SMU40234 accession U40234.1
KEYWORDS    .
SOURCE      Stenotrophomonas maltophilia
  ORGANISM  Stenotrophomonas maltophilia
            Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales;
            Xanthomonadaceae; Stenotrophomonas.
REFERENCE   1  (residues 1 to 264)
  AUTHORS   Lee,N.R., Hwang,M.O., Jung,G.H., Kim,Y.S. and Min,K.H.
  TITLE     Physical structure and expression of alkBA encoding alkane
            hydroxylase and rubredoxin reductase from Pseudomonas maltophilia
  JOURNAL   Biochem. Biophys. Res. Commun. 218 (1), 17-21 (1996)
  MEDLINE   96136269
   PUBMED   8573125
REFERENCE   2  (residues 1 to 264)
  AUTHORS   Min,K.-H.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-NOV-1995) Kyung-Hee Min, Sookmyung Women's
            University, Department of Biology, Chungpa-Dong 2-Ka, Yongsan-Ku,
            Seoul 140-742, Korea
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..264
                     /organism="Stenotrophomonas maltophilia"
                     /strain="N246"
                     /db_xref="taxon:40324"
                     /clone="pPOC122"
                     /clone_lib="library of K.-H. Min"
     Protein         1..264
                     /product="rubredoxin reductase"
     CDS             1..264
                     /gene="alkA"
                     /coded_by="U40234.1:1..>794"
                     /transl_table=11
ORIGIN
        1 mveyycplcv iydtnkgfps fgmhlfswnd teidescpdl mvrlldfkkl spfkftqlgm
       61 glasenlisd ititglansa lalkyplfnf nkieicankt gvllslrlvv eksinklmli
      121 ksasiqlpde levfdfysvs eptptflnpi kwhlpdfsvk ldfildfsgk lvlsgdtphl
      181 sfvilnsqta tvcapftiek lmsatvadlf ktdiprvaqg hiacewrcik mgmydeylty
      241 eafftpgtis idipqdqccy ianv
//
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1: 1606208A. Reports rubredoxin...[gi:226794]


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LOCUS       1606208A                  52 aa            linear   BCT 21-OCT-1996
DEFINITION  rubredoxin.
ACCESSION   1606208A
VERSION     1606208A  GI:226794
DBSOURCE    prf: locus 1606208A;

            state: embryo;
            taxonomy: Prokaryota.
KEYWORDS    Rubredoxin; Desulfovibrio vulgaris; Purifn; LC; CPase Y/A Digest;
            Seq Determination; 52AAs; For-Met at N Term; Seq Comparison; Redox
            Potential of 5mV; Seq Comparison with Cytochrome c.
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Shimizu,F., Ogata,M., Yagi,T., Wakabayashi,S. and Matsubara,H.
  TITLE     Amino acid sequence and function of rubredoxin from Desulfovibrio
            vulgaris Miyazaki
  JOURNAL   Biochimie 71(11/12), 1171-1177 (1989)
COMMENT     N.For:EC=4.1.1.39.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
ORIGIN
        1 mkkyvctvcg yeydpaegdp dngvkpgtaf edvpadwvcp icgapksefe pa
//
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1: 1924243A. Reports rubredoxin...[gi:738104]


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LOCUS       1924243A                75 aa            linear   BCT 21-NOV-1996
DEFINITION  rubredoxin.
ACCESSION   1924243A
VERSION     1924243A  GI:738104
DBSOURCE    prf: locus 1924243A;

            state: embryo;
            taxonomy: Prokaryota.
KEYWORDS    Rubredoxin; Clostridium pasteurianum; Transcription Mapping; Seq
            Determination; ORF 2; Seq Comparison.
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 75)
  AUTHORS   Mathieu,I. and Meyer,J.
  TITLE     Transcript mapping of the rubredoxin gene from Clostridium
            pasteurianum
  JOURNAL   FEMS Microbiol. Lett. 112 (2), 223-227 (1993)
  MEDLINE   94010250
   PUBMED   8405965
COMMENT     EC=5.4.99.5.
FEATURES             Location/Qualifiers
     source          1..75
                     /organism="Clostridium pasteurianum"
                     /db_xref="taxon:1501"
     Region          1..73
                     /region_name="Glutaredoxin"
                     /note="Glutaredoxin"
                     /db_xref="CDD:23000"
ORIGIN
        1 mikiystptc pwckktkeyl ksknidfvdv nvaddmkere emrslskqsg vpvinidgni
       61 ivgfnkaeid kliek
//
```


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1: AAB20233. Reports rubredoxin, Rd [...[gi:238348]


```
LOCUS       AAB20233                 53 aa            linear   BCT 07-MAY-1993
DEFINITION  rubredoxin,  Rd [Pyrococcus furiosus, DSM 3638, Peptide, 53 aa].
ACCESSION   AAB20233
VERSION     AAB20233.1  GI:238348
DBSOURCE    accession AAB20233.1
KEYWORDS    .
SOURCE      Pyrococcus furiosus
  ORGANISM  Pyrococcus furiosus
            Archaea; Euryarchaeota; Thermococci; Thermococcales;
            Thermococcaceae; Pyrococcus.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Blake,P.R., Park,J.B., Bryant,F.O., Aono,S., Magnuson,J.K.,
            Eccleston,E., Howard,J.B., Summers,M.F. and Adams,M.W.
  TITLE     Determinants of protein hyperthermostability: purification and
            amino acid sequence of rubredoxin from the hyperthermophilic
            archaebacterium Pyrococcus furiosus and secondary structure of the
            zinc adduct by NMR
  JOURNAL   Biochemistry 30 (45), 10885-10895 (1991)
  MEDLINE   92031546
   PUBMED   1932012
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 62795] from the original journal article.
COMMENT     Method: direct peptide sequencing.
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Pyrococcus furiosus"
                     /db_xref="taxon:2261"
     Protein         1..53
                     /product="rubredoxin"
                     /note="Rd"
     Region          2..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
ORIGIN
        1 akwvckicgy iydedagdpd ngispgtkfe elpddwvcpi cgapksefek led
//
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1: AAB34046. Reports rubredoxin, Rd=sm...[gi:998902] BLink, Domains, Links

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LOCUS       AAB34046                  52 aa            linear   BCT 27-SEP-1995
DEFINITION  rubredoxin, Rd=small nonheme iron protein [Heliobacillus mobilis,
            Peptide, 52 aa].
ACCESSION   AAB34046
VERSION     AAB34046.1  GI:998902
DBSOURCE    accession AAB34046.1
KEYWORDS    .
SOURCE      Heliobacillus mobilis
  ORGANISM  Heliobacillus mobilis
            Bacteria; Firmicutes; Clostridia; Clostridiales; Heliobacteriaceae;
            Heliobacillus.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Lee,W.Y., Brune,D.C., LoBrutto,R. and Blankenship,R.E.
  TITLE     Isolation, characterization, and primary structure of rubredoxin
            from the photosynthetic bacterium, Heliobacillus mobilis
  JOURNAL   Arch. Biochem. Biophys. 318 (1), 80-88 (1995)
  MEDLINE   95243660
   PUBMED   7726577
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 165993] from the original journal article.
COMMENT     Method: direct peptide sequencing.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Heliobacillus mobilis"
                     /db_xref="taxon:28064"
     Protein         1..52
                     /product="rubredoxin"
                     /name="small nonheme iron protein"
                     /note="Rd"
     Region          3..52
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
ORIGIN
        1 mkkygclvcg yvydpakgdp dhgiapgtaf edlpadwvcp lcgvskdefe pl
//
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☐1: CAA67880. Reports rubredoxin oxydor...[gi:1491677]

BLink, Domains, Links

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LOCUS       CAA67880                 126 aa            linear   BCT 13-DEC-1996
DEFINITION  rubredoxin oxydoreductase [Desulfovibrio baarsii].
ACCESSION   CAA67880
VERSION     CAA67880.1  GI:1491677
DBSOURCE    embl locus DBRBORUB, accession X99543.1
KEYWORDS    .
SOURCE      Desulfovibrio baarsii
  ORGANISM  Desulfovibrio baarsii
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1
  AUTHORS   Pianzzola,M.J., Soubes,M. and Touati,D.
  TITLE     Overproduction of the rbo gene product from Desulfovibrio species
            suppresses all deleterious effects of lack of superoxide dismutase
            in Escherichia coli
  JOURNAL   J. Bacteriol. 178 (23), 6736-6742 (1996)
  MEDLINE   97113430
   PUBMED   8955290
REFERENCE   2
  AUTHORS   Touati,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1996) D. Touati, Institut Jacques Monod,
            Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251
            Paris Cedex 05, FRANCE
  REMARK    revised by [3]
REFERENCE   3  (residues 1 to 126)
  AUTHORS   Touati,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-NOV-1996) D. Touati, Institut Jacques Monod,
            Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251
            Paris Cedex 05, FRANCE
FEATURES             Location/Qualifiers
     source          1..126
                     /organism="Desulfovibrio baarsii"
                     /strain="DSM 2075"
                     /db_xref="taxon:887"
     Protein         1..126
                     /product="rubredoxin oxydoreductase"
     Region          2..37
                     /region_name="Desulfoferrodoxin, N-terminal domain"
                     /note="Desulfoferrod_N"
                     /db_xref="CDD:26510"
     Region          41..125
                     /region_name="Desulfoferrodoxin"
                     /note="Desulfoferrodox"
                     /db_xref="CDD:25838"
     CDS             1..126
                     /gene="rbo"
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                     /coded_by="X99543.1:185..565"
                     /transl_table=11
                     /db_xref="GOA:Q46495"
                     /db_xref="UniProt/Swiss-Prot:Q46495"
ORIGIN
        1 mperlqvykc evcgnivevl nggigelvcc nqdmklmsen tvdaakekhv pviekidggy
       61 kvkvgavahp meekhyiqwi elladdkcyt qflkpgqape avflieaakv vareycnihg
      121 hwkaen
//
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1: CAA67881. Reports rubredoxin [Desul...[gi:1491678] BLink, Domains, Links

```
LOCUS       CAA67881                  53 aa            linear   BCT 13-DEC-1996
DEFINITION  rubredoxin [Desulfovibrio baarsii].
ACCESSION   CAA67881
VERSION     CAA67881.1  GI:1491678
DBSOURCE    embl locus DBRBORUB, accession X99543.1
KEYWORDS    .
SOURCE      Desulfovibrio baarsii
  ORGANISM  Desulfovibrio baarsii
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1
  AUTHORS   Pianzzola,M.J., Soubes,M. and Touati,D.
  TITLE     Overproduction of the rbo gene product from Desulfovibrio species
            suppresses all deleterious effects of lack of superoxide dismutase
            in Escherichia coli
  JOURNAL   J. Bacteriol. 178 (23), 6736-6742 (1996)
  MEDLINE   97113430
   PUBMED   8955290
REFERENCE   2
  AUTHORS   Touati,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1996) D. Touati, Institut Jacques Monod,
            Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251
            Paris Cedex 05, FRANCE
  REMARK    revised by [3]
REFERENCE   3  (residues 1 to 53)
  AUTHORS   Touati,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-NOV-1996) D. Touati, Institut Jacques Monod,
            Microbiologie, Unversite Paris 7, Tour 43, 2 Place Jussieu, 75251
            Paris Cedex 05, FRANCE
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Desulfovibrio baarsii"
                     /strain="DSM 2075"
                     /db_xref="taxon:887"
     Protein         1..53
                     /product="rubredoxin"
     Region          3..50
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     CDS             1..53
                     /gene="rub"
                     /coded_by="X99543.1:615..776"
                     /transl_table=11
                     /db_xref="GOA:Q46496"
                     /db_xref="UniProt/TrEMBL:Q46496"
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ORIGIN
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1: CAA72620. Reports rubredoxin [Clost...[gi:1894774] BLink, Domains, Links

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LOCUS       CAA72620                  53 aa            linear   BCT 17-MAR-1997
DEFINITION  rubredoxin [Clostridium diolis].
ACCESSION   CAA72620
VERSION     CAA72620.1  GI:1894774
DBSOURCE    embl locus CBRUBRDOX, accession Y11875.1
KEYWORDS    .
SOURCE      Clostridium diolis
  ORGANISM  Clostridium diolis
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1
  AUTHORS   Gerard,P.
  JOURNAL   Unpublished
REFERENCE   2  (residues 1 to 53)
  AUTHORS   Gerard,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-MAR-1997) P. Gerard, Universite Henri Poincare,
            Laboratoire de Chimie Biologique, Facult des Sciences, 54506
            Vandoeuvre-Les-Nancy Cdex, BP 239, FRANCE
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Clostridium diolis"
                     /strain="DSM5431"
                     /db_xref="taxon:223919"
     Protein         1..53
                     /product="rubredoxin"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     CDS             1..53
                     /coded_by="Y11875.1:422..583"
                     /transl_table=11
                     /db_xref="GOA:P96745"
                     /db_xref="UniProt/TrEMBL:P96745"
ORIGIN
        1 mekyictvcg yiydeaagdp dngvapgtkf edipddwvcp lcgvpksdfe keq
//
```

1: AAB50346. Reports rubredoxin oxidor...[gi:1905953] BLink, Domains, Links

```
LOCUS       AAB50346                 393 aa            linear   BCT 25-MAR-1997
DEFINITION  rubredoxin oxidoreductase homolog.
ACCESSION   AAB50346
VERSION     AAB50346.1  GI:1905953
DBSOURCE    locus CAU52368 accession U52368.1
KEYWORDS    .
SOURCE      Clostridium acetobutylicum
  ORGANISM  Clostridium acetobutylicum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 393)
  AUTHORS   Belouski,E., Gui,L. and Bennett,G.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-MAR-1996) Ed Belouski, Biochemistry and Cell Biology,
            Rice University, 6100 S. Main, Houston, TX 77005-1892, USA
FEATURES             Location/Qualifiers
     source          1..393
                     /organism="Clostridium acetobutylicum"
                     /strain="ATCC 824"
                     /db_xref="taxon:1488"
     Protein         1..393
                     /product="rubredoxin oxidoreductase homolog"
     Region          <1..392
                     /region_name="Primosomal protein N' (replication factor Y)
                     - superfamily II helicase [DNA replication, recombination,
                     and repair]"
                     /note="PriA"
                     /db_xref="CDD:10916"
     CDS             1..393
                     /coded_by="U52368.1:500..1681"
                     /note="ORFrubr; similar to primosomal protein n' in
                     Haemophilus influenzae, Swiss-Prot Accession Number
                     P44647"
                     /transl_table=11
ORIGIN
        1 mksaiedcil vlgsatpave tyyktlkedy dlitlknrad galmpevsvv dmreelrenn
       61 ksifsralye siletlerke qiilflnrrg ystfvscrkc gyvfkckncd vsmtyhnnkg
      121 ylichycgst qripsvcpkc gskyvkyfga gteriereik rlfpnartvr mdrdttvkkd
      181 syeryimasr tkgydiligt qmiakaldfk dvtlvgvvaa dltlnlpdyr agertfqllc
      241 qvsgragrgs kkgkvviqty spesyairfs stndyrsffk eeiklrhdmd yppfnkllll
      301 nfsskneqll ikniqivgdf lknkakeysn iyvlgpcpsp iskineayrw qivlkgdity
      361 evacfikksi yellkklynn irvsldinpl sll
//
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1: 1RDG. Reports Chain , Rubredox...[gi:230285]

BLink, Domains, Links

```
LOCUS       1RDG                    53 aa            linear   BCT 04-SEP-1998
DEFINITION  Rubredoxin.
ACCESSION   1RDG
VERSION     1RDG  GI:230285
DBSOURCE    pdb: molecule 1RDG, chain 32, release Mar 17, 1988;
            deposition: Mar 17, 1988;
            class: Electron Transfer(Iron-Sulfur Protein);
            source: (Desulfovibrio gigas);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio gigas
  ORGANISM  Desulfovibrio gigas
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
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REFERENCE   1  (residues 1 to 53)
  AUTHORS   Bruschi,M.
  TITLE     The amino acid sequence of rubredoxin from the sulfate reducing
            bacterium, Desulfovibrio gigas
  JOURNAL   Biochem. Biophys. Res. Commun. 70 (2), 615-621 (1976)
  MEDLINE   76231572
   PUBMED   938515
REFERENCE   2  (residues 1 to 53)
  AUTHORS   Pierrot,M., Haser,R., Frey,M., Bruschi,M., le Gall,J., Sieker,L.C.
            and Jensen,L.H.
  TITLE     Some comparisons between two crystallized anaerobic bacterial
            ruberdoxins from Desulfovibrio gigas and D. vulgaris
  JOURNAL   J. Mol. Biol. 107 (2), 179-182 (1976)
  MEDLINE   77074146
   PUBMED   1003467
REFERENCE   3  (residues 1 to 53)
  AUTHORS   Frey,M., Sieker,L., Payan,F., Haser,R., Bruschi,M., Pepe,G. and
            LeGall,J.
  TITLE     Rubredoxin from Desulfovibrio gigas. A molecular model of the
            oxidized form at 1.4 A resolution
  JOURNAL   J. Mol. Biol. 197 (3), 525-541 (1987)
  MEDLINE   88155649
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REFERENCE   4  (residues 1 to 53)
  AUTHORS   Frey,M., Sieker,L.C. and Payan,F.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-MAR-1988)
COMMENT     Revision History:
            JAN 15 91 Typographical
            APR 19 89 Initial Entry.
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Nov 8 2004 13:44:10

1: 4RXN. Reports Chain , Rubredox...[gi:231077] BLink, Domains, Links

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DEFINITION  Rubredoxin (Oxidized, Fe(III)) (Unconstrained Model).
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VERSION     4RXN  GI:231077
DBSOURCE    pdb: molecule 4RXN, chain 32, release Oct 15, 1984;
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            class: Electron Transfer(Iron-Sulfur Protein);
            source: (Clostridium pasteurianum);
            Exp. method: X-Ray Diffraction.
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REFERENCE   1  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.
  TITLE     Structure of rubredoxin: an x-ray study to 2.5 A resolution
  JOURNAL   J. Mol. Biol. 50 (2), 391-406 (1970)
  MEDLINE   71027253
   PUBMED   5476919
REFERENCE   2  (residues 1 to 54)
  AUTHORS   McCarthy,K.F.
  TITLE     The Primary Structure Of Clostridium Pasteurianum Rubredoxin
  JOURNAL   Diss.Abstr.B, Ph.D.Thesis, George Washington University 33, 1436
            (1972)
REFERENCE   3  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     The structure of a non-heme iron protein: rubredoxin at 1.5
            Angstrom resolution
  JOURNAL   Cold Spring Harb. Symp. Quant. Biol. 36, 359-367 (1972)
  MEDLINE   73041148
   PUBMED   4508149
REFERENCE   4  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms
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  JOURNAL   Acta Crystallogr.,Sect.B 29, 943 (1973)
REFERENCE   5  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Sequence of rubredoxin by x-ray diffraction
  JOURNAL   J. Mol. Biol. 80 (3), 423-432 (1973)
  MEDLINE   74053319
   PUBMED   4762562
REFERENCE   6  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Margulis,T.N., Sieker,L.C. and Jensen,L.H.
  TITLE     Water structure in a protein crystal: rubredoxin at 1.2 A
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  JOURNAL   J. Mol. Biol. 122 (2), 175-190 (1978)
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  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     The structure of rubredoxin at 1.2 A resolution
  JOURNAL   J. Mol. Biol. 131 (3), 509-522 (1979)
  MEDLINE   80074980
   PUBMED   513127
REFERENCE   8  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Crystallographic refinement of rubredoxin at 1 x 2 A degrees
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  JOURNAL   J. Mol. Biol. 138 (3), 615-633 (1980)
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REFERENCE   9  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-OCT-1984)
COMMENT     Revision History:
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            APR 1 85 Initial Entry.
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Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search Protein for Go Clear

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1: 5RXN. Reports Chain , Rubredox...[gi:231175] BLink, Domains, Links

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DEFINITION  Rubredoxin (Oxidized, Fe(III)) (Constrained Model).
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VERSION     5RXN  GI:231175
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            deposition: Oct 15, 1984;
            class: Electron Transfer(Iron-Sulfur Protein);
            source: (Clostridium pasteurianum);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
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            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.
  TITLE     Structure of rubredoxin: an x-ray study to 2.5 A resolution
  JOURNAL   J. Mol. Biol. 50 (2), 391-406 (1970)
  MEDLINE   71027253
   PUBMED   5476919
REFERENCE   2  (residues 1 to 54)
  AUTHORS   McCarthy,K.F.
  TITLE     The Primary Structure Of Clostridium Pasteurianum Rubredoxin
  JOURNAL   Diss.Abstr.B, Ph.D.Thesis, George Washington University 33, 1436
            (1972)
REFERENCE   3  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     The structure of a non-heme iron protein: rubredoxin at 1.5
            Angstrom resolution
  JOURNAL   Cold Spring Harb. Symp. Quant. Biol. 36, 359-367 (1972)
  MEDLINE   73041148
   PUBMED   4508149
REFERENCE   4  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms
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  JOURNAL   Acta Crystallogr.,Sect.B 29, 943 (1973)
REFERENCE   5  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Sequence of rubredoxin by x-ray diffraction
  JOURNAL   J. Mol. Biol. 80 (3), 423-432 (1973)
  MEDLINE   74053319
   PUBMED   4762562
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  AUTHORS   Watenpaugh,K.D., Margulis,T.N., Sieker,L.C. and Jensen,L.H.
  TITLE     Water structure in a protein crystal: rubredoxin at 1.2 A
            resolution
  JOURNAL   J. Mol. Biol. 122 (2), 175-190 (1978)
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REFERENCE   7  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     The structure of rubredoxin at 1.2 A resolution
  JOURNAL   J. Mol. Biol. 131 (3), 509-522 (1979)
  MEDLINE   80074980
   PUBMED   513127
REFERENCE   8  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Crystallographic refinement of rubredoxin at 1 x 2 A degrees
            resolution
  JOURNAL   J. Mol. Biol. 138 (3), 615-633 (1980)
  MEDLINE   81009589
   PUBMED   7411618
REFERENCE   9  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D.
  TITLE     Combined Crystallographic Refinement And Energy Minimization Of
            Rubredoxin At 1.2 Angstrom Resolution
  JOURNAL   Unpublished
REFERENCE   10 (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-OCT-1984)
COMMENT     Revision History:
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            APR 1 85 Initial Entry.
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            class: Electron Transfer(Iron-Sulfur Protein);
            source: (Desulfovibrio desulfuricans, Strain 27774);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio desulfuricans
  ORGANISM  Desulfovibrio desulfuricans
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
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  AUTHORS   Sieker,L.C., Jensen,L.H., Prickril,B.C. and LeGall,J.
  TITLE     Crystallographic study of rubredoxin from the bacterium
            Desulfovibrio desulfuricans strain 27774
  JOURNAL   J. Mol. Biol. 171 (1), 101-103 (1983)
  MEDLINE   84064867
   PUBMED   6644818
REFERENCE   2  (residues 1 to 46)
  AUTHORS   Sieker,L.C., Stenkamp,R.E., Jensen,L.H., Prickril,B. and LeGall,J.
  TITLE     Structure of rubredoxin from the bacterium Desulfovibrio
            desulfuricans
  JOURNAL   FEBS Lett. 208 (1), 73-76 (1986)
  MEDLINE   87030959
   PUBMED   3770211
REFERENCE   3  (residues 1 to 46)
  AUTHORS   Stenkamp,R.E., Sieker,L.C. and Jensen,L.H.
  TITLE     The Structure Of Rubredoxin From Desulfovibrio desulfuricans
  JOURNAL   Unpublished
REFERENCE   4  (residues 1 to 46)
  AUTHORS   Stenkamp,R.E., Sieker,L.C. and Jensen,L.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JAN-1990)
COMMENT     Revision History:
            JUL 15 91 Coordinates
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1: 7RXN. Reports Chain , Rubredox...[gi:231271] BLink, Domains, Links

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DEFINITION  Rubredoxin.
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VERSION     7RXN  GI:231271
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            class: Electron Transfer(Iron-Sulfur Protein);
            source: (Desulfovibrio vulgaris);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Adman,E.T., Sieker,L.C., Jensen,L.H., Bruschi,M. and Le Gall,J.
  TITLE     A structural model of rubredoxin from Desulfovibrio vulgaris at 2 A
            resolution
  JOURNAL   J. Mol. Biol. 112 (1), 113-120 (1977)
  MEDLINE   77230456
   PUBMED   881725
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Adman,E.T. and Jensen,L.H.
  TITLE     Progress On Refinement Of Rubredoxin (D. Vulgaris) At 1.5 Angstroms
  JOURNAL   Am.Cryst.Assoc.,Abstr.Papers (Winter Meeting) 6, 65 (1979)
REFERENCE   3  (residues 1 to 52)
  AUTHORS   Adman,E.T., Sieker,L.C. and Jensen,L.H.
  TITLE     Structure of rubredoxin from Desulfovibrio vulgaris at 1.5 A
            resolution
  JOURNAL   J. Mol. Biol. 217 (2), 337-352 (1991)
  MEDLINE   91124457
   PUBMED   1992166
REFERENCE   4  (residues 1 to 52)
  AUTHORS   Adman,E.T., Sieker,L.C. and Jensen,L.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-MAY-1990)
COMMENT     Revision History:
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            JUL 15 91 Initial Entry.
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1: 1CAA. Reports Chain , Rubredox...[gi:442697]

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VERSION     1CAA  GI:442697
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            class: Electron Transport;
            source: (Pyrococcus Furiosus);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Pyrococcus furiosus
  ORGANISM  Pyrococcus furiosus
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REFERENCE   1  (residues 1 to 53)
  AUTHORS   Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H., Adams,M.W.
            and Rees,D.C.
  TITLE     X-ray crystal structures of the oxidized and reduced forms of the
            rubredoxin from the marine hyperthermophilic archaebacterium
            Pyrococcus furiosus
  JOURNAL   Protein Sci. 1 (11), 1494-1507 (1992)
  MEDLINE   93271899
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REFERENCE   2  (residues 1 to 53)
  AUTHORS   Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H.,
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  TITLE     Direct Submission
  JOURNAL   Submitted (18-MAY-1992)
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            OCT 31 93 Initial Entry.
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DEFINITION  Rubredoxin (Reduced).
ACCESSION   1CAD
VERSION     1CAD  GI:442698
DBSOURCE    pdb: molecule 1CAD, chain 32, release May 18, 1992;
            deposition: May 18, 1992;
            class: Electron Transport;
            source: (Pyrococcus Furiosus);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Pyrococcus furiosus
  ORGANISM  Pyrococcus furiosus
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            Thermococcaceae; Pyrococcus.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H., Adams,M.W.
            and Rees,D.C.
  TITLE     X-ray crystal structures of the oxidized and reduced forms of the
            rubredoxin from the marine hyperthermophilic archaebacterium
            Pyrococcus furiosus
  JOURNAL   Protein Sci. 1 (11), 1494-1507 (1992)
  MEDLINE   93271899
   PUBMED   1303768
REFERENCE   2  (residues 1 to 53)
  AUTHORS   Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B., Zhou,Z.H.,
            Adams,M.W.W. and Rees,D.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-MAY-1992)
COMMENT     Revision History:
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DEFINITION  Rubredoxin (Zn-Substituted) (Nmr, 40 Structures).
ACCESSION   1ZRP
VERSION     1ZRP  GI:443341
DBSOURCE    pdb: molecule 1ZRP, chain 32, release Jul 10, 1992;
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            Exp. method: Nmr.
KEYWORDS    .
SOURCE      Pyrococcus furiosus
  ORGANISM  Pyrococcus furiosus
            Archaea; Euryarchaeota; Thermococci; Thermococcales;
            Thermococcaceae; Pyrococcus.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Blake,P.R., Park,J.B., Bryant,F.O., Aono,S., Magnuson,J.K.,
            Eccleston,E., Howard,J.B., Summers,M.F. and Adams,M.W.
  TITLE     Determinants of protein hyperthermostability: purification and
            amino acid sequence of rubredoxin from the hyperthermophilic
            archaebacterium Pyrococcus furiosus and secondary structure of the
            zinc adduct by NMR
  JOURNAL   Biochemistry 30 (45), 10885-10895 (1991)
  MEDLINE   92031546
   PUBMED   1932012
REFERENCE   2  (residues 1 to 53)
  AUTHORS   Blake,P.R., Park,J.B., Adams,M.W.W. and Summers,M.F.
  TITLE     Novel Observation Of Nh...S(Cys) Hydrogen-Bond-Mediated Scalar
            Coupling In 113Cd-Substituted Rubredoxin From Pyrococcus Furiosus
  JOURNAL   J.Am.Chem.Soc. 114, 4931 (1992)
REFERENCE   3  (residues 1 to 53)
  AUTHORS   Blake,P.R., Lee,B., Summers,M.F., Adams,M.W., Park,J.B., Zhou,Z.H.
            and Bax,A.
  TITLE     Quantitative measurement of small through-hydrogen-bond and
            'through-space' 1H-113Cd and 1H-199Hg J couplings in
            metal-substituted rubredoxin from Pyrococcus furiosus
  JOURNAL   J. Biomol. NMR 2 (5), 527-533 (1992)
  MEDLINE   93044392
   PUBMED   1422158
REFERENCE   4  (residues 1 to 53)
  AUTHORS   Blake,P.R., Park,J.B., Zhou,Z.H., Hare,D.R., Adams,M.W. and
            Summers,M.F.
  TITLE     Solution-state structure by NMR of zinc-substituted rubredoxin from
            the marine hyperthermophilic archaebacterium Pyrococcus furiosus
  JOURNAL   Protein Sci. 1 (11), 1508-1521 (1992)
  MEDLINE   93271900
   PUBMED   1303769
REFERENCE   5  (residues 1 to 53)
  AUTHORS   Blake,P.R., Day,M.W., Hsu,B.T., Joshua-Tor,L., Park,J.B.,
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            Hare,D.R., Adams,M.W., Rees,D.C. and Summers,M.F.
  TITLE     Comparison of the X-ray structure of native rubredoxin from
            Pyrococcus furiosus with the NMR structure of the zinc-substituted
            protein
  JOURNAL   Protein Sci. 1 (11), 1522-1525 (1992)
  MEDLINE   93271901
   PUBMED   1303770
REFERENCE   6  (residues 1 to 53)
  AUTHORS   Blake,P.R., Park,J.B., Zhou,Z.H., Hare,D.R., Adams,M.W.W. and
            Summers,M.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-JUL-1992)
COMMENT     Revision History:
            OCT 31 93 Initial Entry.
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1: 8RXNA. Reports Chain A, Rubredox...[gi:443567]

BLink, Domains, Links

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ACCESSION   8RXN_A
VERSION     8RXN_A  GI:443567
DBSOURCE    pdb: molecule 8RXN, chain 65, release Aug 26, 1991;
            deposition: Aug 26, 1991;
            class: Electron Transport(Iron);
            source: (Desulfovibrio Vulgaris);
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Dauter,Z., Sieker,L.C. and Wilson,K.S.
  TITLE     Refinement of rubredoxin from Desulfovibrio vulgaris at 1.0 A with
            and without restraints
  JOURNAL   Acta Crystallogr., B 48 (Pt 1), 42-59 (1992)
  MEDLINE   92313601
   PUBMED   1616692
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Dauter,Z., Sieker,L. and Wilson,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-AUG-1991)
COMMENT     Revision History:
            OCT 31 93 Initial Entry.
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     SecStr          24..27
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1: 1RB9. Reports Chain , Rubredox...[gi:4558184]


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LOCUS       1RB9                      53 aa            linear   BCT 21-DEC-1997
DEFINITION  Rubredoxin From Desulfovibrio Vulgaris Refined Anisotropically At
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ACCESSION   1RB9
VERSION     1RB9  GI:4558184
DBSOURCE    pdb: molecule 1RB9, chain 32, release Dec 21, 1997;
            deposition: Dec 21, 1997;
            class: Iron-Sulfur Protein;
            source: Mol_id: 1; Organism_scientific: Desulfovibrio Vulgaris;
            Strain: Hildenborough; Expression_system: Escherichia Coli;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Dauter,Z., Butterworth,S., Sieker,L.C., Sheldrick,G. and
            Wilson,K.S.
  TITLE     Anisotropic Refinement Of Rubredoxin From Desulfovibrio Vulgaris
  JOURNAL   Unpublished
REFERENCE   2  (residues 1 to 53)
  AUTHORS   Dauter,Z., Butterworth,S., Sieker,L.C., Sheldrick,G. and
            Wilson,K.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-DEC-1997)
COMMENT     Revision History:
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                     /db_xref="taxon:881"
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                     /db_xref="CDD:24175"
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                     /note="strand 1"
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                     bond(43),bond(43))
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Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

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☐1: 1BE7. Reports Chain , Clostrid...[gi:3659994]

BLink, Domains, Links

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LOCUS       1BE7                      54 aa            linear   BCT 20-MAY-1998
DEFINITION  Clostridium Pasteurianum Rubredoxin C42s Mutant.
ACCESSION   1BE7
VERSION     1BE7  GI:3659994
DBSOURCE    pdb: molecule 1BE7, chain 32, release May 20, 1998;
            deposition: May 20, 1998;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Cellular_location: Cytoplasm; Expression_system: Escherichia Coli;
            Expression_system_strain: Jm109;
            Expression_system_cellular_location: Cytoplasm;
            Expression_system_vector_type: Plasmid; Expression_system_plasmid:
            Pkk223-3; Expression_system_gene: Clorub;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Xiao,Z., Lavery,M.J., Ayhan,M., Scrofani,S.D.B., Wilce,M.C.J.,
            Guss,J.M., Tregloan,P.A., George,G.N. and Wedd,A.G.
  TITLE     The Rubredoxin From Clostridium Pasteurianum: Mutation Of The Iron
            Cysteinyl Ligands To Serine. Crystal And Molecular Structures Of
            The Oxidised And Dithionite-Treated Forms Of The Cys42ser Mutant
  JOURNAL   J.Am.Chem.Soc. 120, 4135 (1998)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Maher,M., Guss,J.M., Wilce,M. and Wedd,A.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-MAY-1998)
COMMENT     Revision History:
            SEP 23 98 Initial Entry.
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1501"
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1: 1B13A. Reports Chain A, Clostrid...[gi:5107456] BLink, Domains, Links

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LOCUS       1B13_A                   54 aa            linear   BCT 26-NOV-1998
DEFINITION  Chain A, Clostridium Pasteurianum Rubredoxin G10a Mutant.
ACCESSION   1B13_A
VERSION     1B13_A  GI:5107456
DBSOURCE    pdb: molecule 1B13, chain 65, release Nov 26, 1998;
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            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Strain: Jm109; Cellular_location: Cytoplasm; Gene: Clorub;
            Expression_system: Escherichia Coli; Expression_system_strain:
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            Expression_system_plasmid: Pkk223-3; Expression_system_gene:
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            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.
  TITLE     Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a
            And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To
            Valine Causes The 9-10 Peptide Link To Invert
  JOURNAL   Acta Crystallogr., Sect.D, 962 (1999)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-NOV-1998)
COMMENT     Revision History:
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1: 1B2JA. Reports Chain A, Clostrid...[gi:5107457] BLink, Domains, Links

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LOCUS       1B2J_A                  54 aa            linear   BCT 27-NOV-1998
DEFINITION  Chain A, Clostridium Pasteurianum Rubredoxin G43a Mutant.
ACCESSION   1B2J_A
VERSION     1B2J_A  GI:5107457
DBSOURCE    pdb: molecule 1B2J, chain 65, release Nov 27, 1998;
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            class: Electron Transport;
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            Expression_system: Escherichia Coli; Expression_system_strain:
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            Expression_system_plasmid: Pkk223-3; Expression_system_gene:
            Clorub;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.
  TITLE     Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a
            And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To
            Valine Causes The 9-10 Peptide Link To Invert
  JOURNAL   Acta Crystallogr., Sect.D, 962 (1999)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-NOV-1998)
COMMENT     Revision History:
            MAY 27 99 Initial Entry.
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1: 1B2OA. Reports Chain A, Clostrid...[gi:5107458] BLink, Domains, Links

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DEFINITION  Chain A, Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.
ACCESSION   1B2O_A
VERSION     1B2O_A  GI:5107458
DBSOURCE    pdb: molecule 1B2O, chain 65, release Nov 30, 1998;
            deposition: Nov 30, 1998;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Strain: Jm109; Cellular_location: Cytoplasm; Gene: Clorub;
            Expression_system: Escherichia Coli; Expression_system_strain:
            Jm109; Expression_system_vector_type: Plasmid;
            Expression_system_plasmid: Pkk223-3; Expression_system_gene:
            Clorub;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
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            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.
  TITLE     Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a
            And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To
            Valine Causes The 9-10 Peptide Link To Invert
  JOURNAL   Acta Crystallogr., Sect.D, 962 (1999)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-NOV-1998)
COMMENT     Revision History:
            MAY 27 99 Initial Entry.
FEATURES             Location/Qualifiers
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1: 1B2OB. Reports Chain B, Clostrid...[gi:5107459]

BLink, Domains, Links

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LOCUS       1B2O_B                    54 aa            linear   BCT 30-NOV-1998
DEFINITION  Chain B, Clostridium Pasteurianum Rubredoxin G10vg43a Mutant.
ACCESSION   1B2O_B
VERSION     1B2O_B  GI:5107459
DBSOURCE    pdb: molecule 1B2O, chain 66, release Nov 30, 1998;
            deposition: Nov 30, 1998;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Strain: Jm109; Cellular_location: Cytoplasm; Gene: Clorub;
            Expression_system: Escherichia Coli; Expression_system_strain:
            Jm109; Expression_system_vector_type: Plasmid;
            Expression_system_plasmid: Pkk223-3; Expression_system_gene:
            Clorub;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Maher,M.J., Xiao,Z., Wilce,M.C.J., Guss,J.M. and Wedd,A.G.
  TITLE     Rubredoxin From Clostridium Pasteurianum. Structures Of G10a, G43a
            And G10vg43a Mutant Proteins. Mutation Of Conserved Glycine 10 To
            Valine Causes The 9-10 Peptide Link To Invert
  JOURNAL   Acta Crystallogr., Sect.D, 962 (1999)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Maher,M.J., Guss,J.M., Wilce,M.C.J. and Wedd,A.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-NOV-1998)
COMMENT     Revision History:
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FEATURES             Location/Qualifiers
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Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

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1: 1BFY. Reports Chain , Solution...[gi:5107503]

BLink, Domains, Links

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LOCUS       1BFY                       54 aa            linear   BCT 23-MAY-1998
DEFINITION  Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin,
            Nmr, 20 Structures.
ACCESSION   1BFY
VERSION     1BFY  GI:5107503
DBSOURCE    pdb: molecule 1BFY, chain 32, release May 23, 1998;
            deposition: May 23, 1998;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Exp. method: Nmr, 20 Structures.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Bertini,I., Kurtz,D.M. Jr., Eidsness,M.K., Liu,G., Luchinat,C.,
            Rosato,A. and Scott,R.A.
  TITLE     Solution Structure Of Reduced Clostridium Pasteurianum Rubredoxin
  JOURNAL   Jbic, J.Biol.Inorg.Chem. 3, 401 (1998)
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Bertini,I., Kurtz,D.M. Jr., Eidsness,M.K., Liu,G., Luchinat,C.,
            Rosato,A. and Scott,R.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-MAY-1998)
COMMENT     Revision History:
            MAY 25 99 Initial Entry.
FEATURES             Location/Qualifiers
     source          1..54
                     /organism="Clostridium pasteurianum"
                     /db_xref="taxon:1501"
     Region          3..52
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     SecStr          3..7
                     /sec_str_type="sheet"
                     /note="strand 1"
     Het             join(bond(6),bond(9),bond(39),bond(42))
                     /heterogen="( FE,  55 )"
     SecStr          11..14
                     /sec_str_type="sheet"
                     /note="strand 2"
     SecStr          48..52
                     /sec_str_type="sheet"
                     /note="strand 3"
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1: 1RDV. Reports Chain , Rubredox...[gi:5107680] BLink, Domains, Links

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LOCUS       1RDV                    52 aa            linear   BCT 30-SEP-1998
DEFINITION  Rubredoxin From Desulfovibrio Vulgaris Miyazaki F, Trigonal Crystal
            Form.
ACCESSION   1RDV
VERSION     1RDV  GI:5107680
DBSOURCE    pdb: molecule 1RDV, chain 32, release Sep 30, 1998;
            deposition: Sep 30, 1998;
            class: Electron Transfer;
            source: Mol_id: 1; Organism_scientific: Desulfovibrio Vulgaris;
            Strain: Miyazaki F; Other_details: Iam 12604;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and
            Yasuoka,T.
  TITLE     Preliminary Crystallographic Study Of Two Crystal Forms Of
            Rubredoxin From Sulfate-Reducing Bacterium
  JOURNAL   Protein Pept.Lett. 5, 175 (1998)
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and
            Yasuoka,N.
  TITLE     Structure determination of rubredoxin from Desulfovibrio vulgaris
            Miyazaki F in two crystal forms
  JOURNAL   Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)
  MEDLINE   99190893
   PUBMED   10089348
REFERENCE   3  (residues 1 to 52)
  AUTHORS   Higuchi,Y. and Yasuoka,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-1998)
COMMENT     Revision History:
            MAY 18 99 Initial Entry.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     SecStr          3..8
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                     /note="strand 1"
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                     /note="strand 3"
     SecStr          23..27
                     /sec_str_type="sheet"
                     /note="strand 4"
     SecStr          48..51
                     /sec_str_type="sheet"
                     /note="strand 5"
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1: 2RDVA. Reports Chain A, Rubredox...[gi:5107733] BLink, Domains, Links

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LOCUS       2RDV_A                    52 aa            linear   BCT 07-OCT-1998
DEFINITION  Chain A, Rubredoxin From Desulfovibrio Vulgaris Miyazaki F,
            Monoclinic Crystal Form.
ACCESSION   2RDV_A
VERSION     2RDV_A  GI:5107733
DBSOURCE    pdb: molecule 2RDV, chain 65, release Oct 7, 1998;
            deposition: Oct 7, 1998;
            class: Electron Transfer;
            source: Mol_id: 1; Organism_scientific: Desulfovibrio Vulgaris;
            Strain: Miyazaki F; Other_details: Iam 12604;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and
            Yasuoka,T.
  TITLE     Preliminary Crystallographic Study Of Two Crystal Forms Of
            Rubredoxin From Sulfate-Reducing Bactrium
  JOURNAL   Protein Pept.Lett. 5, 175 (1998)
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and
            Yasuoka,N.
  TITLE     Structure determination of rubredoxin from Desulfovibrio vulgaris
            Miyazaki F in two crystal forms
  JOURNAL   Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)
  MEDLINE   99190893
   PUBMED   10089348
REFERENCE   3  (residues 1 to 52)
  AUTHORS   Higuchi,Y. and Yasuoka,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998)
COMMENT     Revision History:
            MAY 18 99 Initial Entry.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     SecStr          3..8
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                     /note="strand 1"
     Het             join(bond(6),bond(9),bond(39),bond(42))
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                         /sec_str_type="sheet"
                         /note="strand 2"
     SecStr              16..21
                         /sec_str_type="sheet"
                         /note="strand 3"
     SecStr              22..27
                         /sec_str_type="sheet"
                         /note="strand 4"
     SecStr              48..52
                         /sec_str_type="sheet"
                         /note="strand 5"
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1: 2RDVB. Reports Chain B, Rubredox...[gi:5107734] BLink, Domains, Links

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LOCUS       2RDV_B                    52 aa            linear   BCT 07-OCT-1998
DEFINITION  Chain B, Rubredoxin From Desulfovibrio Vulgaris Miyazaki F,
            Monoclinic Crystal Form.
ACCESSION   2RDV_B
VERSION     2RDV_B  GI:5107734
DBSOURCE    pdb: molecule 2RDV, chain 66, release Oct 7, 1998;
            deposition: Oct 7, 1998;
            class: Electron Transfer;
            source: Mol_id: 1; Organism_scientific: Desulfovibrio Vulgaris;
            Strain: Miyazaki F; Other_details: Iam 12604;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and
            Yasuoka,T.
  TITLE     Preliminary Crystallographic Study Of Two Crystal Forms Of
            Rubredoxin From Sulfate-Reducing Bactrium
  JOURNAL   Protein Pept.Lett. 5, 175 (1998)
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and
            Yasuoka,N.
  TITLE     Structure determination of rubredoxin from Desulfovibrio vulgaris
            Miyazaki F in two crystal forms
  JOURNAL   Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)
  MEDLINE   99190893
   PUBMED   10089348
REFERENCE   3  (residues 1 to 52)
  AUTHORS   Higuchi,Y. and Yasuoka,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998)
COMMENT     Revision History:
            MAY 18 99 Initial Entry.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     SecStr          3..8
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                     /note="strand 7"
     SecStr          16..19
                     /sec_str_type="sheet"
                     /note="strand 8"
     SecStr          24..27
                     /sec_str_type="sheet"
                     /note="strand 9"
     SecStr          48..51
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                     /note="strand 10"
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1: 2RDVC. Reports Chain C, Rubredox...[gi:5107735] BLink, Domains, Links

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LOCUS       2RDV_C                  52 aa            linear   BCT 07-OCT-1998
DEFINITION  Chain C, Rubredoxin From Desulfovibrio Vulgaris Miyazaki F,
            Monoclinic Crystal Form.
ACCESSION   2RDV_C
VERSION     2RDV_C  GI:5107735
DBSOURCE    pdb: molecule 2RDV, chain 67, release Oct 7, 1998;
            deposition: Oct 7, 1998;
            class: Electron Transfer;
            source: Mol_id: 1; Organism_scientific: Desulfovibrio Vulgaris;
            Strain: Miyazaki F; Other_details: Iam 12604;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Higuchi,Y., Sugiyama,M.S., Morimoto,S., Ogata,Y., Yagi,M. and
            Yasuoka,T.
  TITLE     Preliminary Crystallographic Study Of Two Crystal Forms Of
            Rubredoxin From Sulfate-Reducing Bactrium
  JOURNAL   Protein Pept.Lett. 5, 175 (1998)
REFERENCE   2  (residues 1 to 52)
  AUTHORS   Misaki,S., Morimoto,Y., Ogata,M., Yagi,T., Higuchi,Y. and
            Yasuoka,N.
  TITLE     Structure determination of rubredoxin from Desulfovibrio vulgaris
            Miyazaki F in two crystal forms
  JOURNAL   Acta Crystallogr D Biol Crystallogr 55 (Pt 2), 408-413 (1999)
  MEDLINE   99190893
   PUBMED   10089348
REFERENCE   3  (residues 1 to 52)
  AUTHORS   Higuchi,Y. and Yasuoka,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998)
COMMENT     Revision History:
            MAY 18 99 Initial Entry.
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Region          3..51
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                     /note="rubredoxin"
                     /db_xref="CDD:24175"
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                     /note="strand 13"
     SecStr          24..27
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     SecStr          48..52
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☐1: 1IRN. Reports Chain , Rubredox...[gi:1421138]

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LOCUS       1IRN                    54 aa            linear   BCT 13-DEC-1995
DEFINITION  Rubredoxin (Zn-Substituted) At 1.2 Angstroms Resolution.
ACCESSION   1IRN
VERSION     1IRN  GI:1421138
DBSOURCE    pdb: molecule 1IRN, chain 32, release Dec 13, 1995;
            deposition: Dec 13, 1995;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.
  TITLE     Structure of rubredoxin: an x-ray study to 2.5 A resolution
  JOURNAL   J. Mol. Biol. 50 (2), 391-406 (1970)
  MEDLINE   71027253
   PUBMED   5476919
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms
            Resolution
  JOURNAL   Acta Crystallogr.,Sect.B 29, 943 (1973)
REFERENCE   3  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Crystallographic refinement of rubredoxin at 1 x 2 A degrees
            resolution
  JOURNAL   J. Mol. Biol. 138 (3), 615-633 (1980)
  MEDLINE   81009589
   PUBMED   7411618
REFERENCE   4  (residues 1 to 54)
  AUTHORS   Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.
  TITLE     Zinc- and iron-rubredoxins from Clostridium pasteurianum at atomic
            resolution: a high-precision model of a ZnS4 coordination unit in a
            protein
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 93 (17), 8836-8840 (1996)
  MEDLINE   96392325
   PUBMED   8799113
REFERENCE   5  (residues 1 to 54)
  AUTHORS   Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-DEC-1995)
COMMENT     Revision History:
            JAN 5 0 Typographical
            APR 3 96 Initial Entry.
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1501"
     Region          3..52
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                     /note="rubredoxin"
                     /db_xref="CDD:24175"
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                     /sec_str_type="sheet"
                     /note="strand 1"
     Het             join(bond(6),bond(9),bond(39),bond(42))
                     /heterogen="( ZN,   1 ) Zinc Ion"
     SecStr          9..14
                     /sec_str_type="sheet"
                     /note="strand 2"
     SecStr          16..19
                     /sec_str_type="sheet"
                     /note="strand 3"
     SecStr          24..27
                     /sec_str_type="sheet"
                     /note="strand 4"
     SecStr          48..52
                     /sec_str_type="sheet"
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BLink, Domains, Links

☐1: 1IRO. Reports Chain , Rubredox...[gi:1421143]

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LOCUS       1IRO                      54 aa            linear   BCT 13-DEC-1995
DEFINITION  Rubredoxin (Oxidized, Fe(Iii)) At 1.1 Angstroms Resolution.
ACCESSION   1IRO
VERSION     1IRO  GI:1421143
DBSOURCE    pdb: molecule 1IRO, chain 32, release Dec 13, 1995;
            deposition: Dec 13, 1995;
            class: Electron Transport;
            source: Mol_id: 1; Organism_scientific: Clostridium Pasteurianum;
            Exp. method: X-Ray Diffraction.
KEYWORDS    .
SOURCE      Clostridium pasteurianum
  ORGANISM  Clostridium pasteurianum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Herriott,J.R., Sieker,L.C., Jensen,L.H. and Lovenberg,W.
  TITLE     Structure of rubredoxin: an x-ray study to 2.5 A resolution
  JOURNAL   J. Mol. Biol. 50 (2), 391-406 (1970)
  MEDLINE   71027253
   PUBMED   5476919
REFERENCE   2  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C., Herriott,J.R. and Jensen,L.H.
  TITLE     Refinement Of The Model Of A Protein. Rubredoxin At 1.5 Angstroms
            Resolution
  JOURNAL   Acta Crystallogr.,Sect.B 29, 943 (1973)
REFERENCE   3  (residues 1 to 54)
  AUTHORS   Watenpaugh,K.D., Sieker,L.C. and Jensen,L.H.
  TITLE     Crystallographic refinement of rubredoxin at 1 x 2 A degrees
            resolution
  JOURNAL   J. Mol. Biol. 138 (3), 615-633 (1980)
  MEDLINE   81009589
   PUBMED   7411618
REFERENCE   4  (residues 1 to 54)
  AUTHORS   Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.
  TITLE     Zinc- and iron-rubredoxins from Clostridium pasteurianum at atomic
            resolution: a high-precision model of a ZnS4 coordination unit in a
            protein
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 93 (17), 8836-8840 (1996)
  MEDLINE   96392325
   PUBMED   8799113
REFERENCE   5  (residues 1 to 54)
  AUTHORS   Dauter,Z., Wilson,K.S., Sieker,L.C., Moulis,J.M. and Meyer,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-DEC-1995)
COMMENT     Revision History:
            JAN 5 0 Typographical
            APR 3 96 Initial Entry.
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1501"
     Region          3..52
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     SecStr          3..8
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     Het             join(bond(6),bond(9),bond(39),bond(42))
                     /heterogen="( FE,   1 ) Fe(Iii) Ion"
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                     /note="strand 2"
     SecStr          16..19
                     /sec_str_type="sheet"
                     /note="strand 3"
     SecStr          24..27
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                     /note="strand 4"
     SecStr          48..52
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                     /note="strand 5"
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1: RUPE. Reports rubredoxin - Pept...[gi:65797] BLink, Domains, Links

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LOCUS       RUPE                        53 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - Peptostreptococcus asaccharolyticus.
ACCESSION   RUPE
VERSION     RUPE  GI:65797
DBSOURCE    pir: locus RUPE;

            summary: #length 53 #molecular-weight 5911 #checksum 6231
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 13-Jul-1981 #sequence_revision 13-Jul-1981 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Peptoniphilus asaccharolyticus
  ORGANISM  Peptoniphilus asaccharolyticus
            Bacteria; Firmicutes; Clostridia; Clostridiales;
            Peptostreptococcaceae; Peptoniphilus.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Bachmayer,H., Benson,A.M., Yasunobu,K.T., Garrard,W.T. and
            Whiteley,H.R.
  TITLE     Nonheme iron proteins. IV. Structural studies of Micrococcus
            aerogenes rubredoxin
  JOURNAL   Biochemistry 7 (3), 986-996 (1968)
  MEDLINE   68311179
   PUBMED   5657864
FEATURES             Location/Qualifiers
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                     /organism="Peptoniphilus asaccharolyticus"
                     /db_xref="taxon:1258"
     Protein         1..53
                     /product="rubredoxin"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     Region          3..48
                     /region_name="domain"
                     /note="rubredoxin homology #label RUB"
     Site            6
                     /site_type="binding"
                     /note="iron (Cys)"
     Site            9
                     /site_type="binding"
                     /note="iron (Cys)"
     Site            38
                     /site_type="binding"
                     /note="iron (Cys)"
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     Site            41
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1: A27537. Reports rubredoxin - Chlo...[gi:79592] BLink, Domains, Links

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LOCUS       A27537                   53 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - Chlorobium limicola f.sp. thiosulfatophilum.
ACCESSION   A27537
VERSION     A27537  GI:79592
DBSOURCE    pir: locus A27537;

            summary: #length 53 #molecular-weight 5843 #checksum 6847
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 05-Jun-1988 #sequence_revision 22-Jul-1994 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Chlorobaculum thiosulfatiphilum
  ORGANISM  Chlorobaculum thiosulfatiphilum
            Bacteria; Chlorobi; Chlorobia; Chlorobiales; Chlorobiaceae;
            Chlorobaculum.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Woolley,K.J. and Meyer,T.E.
  TITLE     The complete amino acid sequence of rubredoxin from the green
            phototrophic bacterium Chlorobium thiosulphatophilum strain PM
  JOURNAL   Eur. J. Biochem. 163 (1), 161-166 (1987)
  MEDLINE   87133563
   PUBMED   3816795
  REMARK    Chlorobium thiosulphatophilum
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Chlorobaculum thiosulfatiphilum"
                     /db_xref="taxon:115852"
                     /note="forma: thiosulfatophilum"
     Protein         1..53
                     /product="rubredoxin"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     Region          3..49
                     /region_name="domain"
                     /note="rubredoxin homology #label RUB"
     Site            6
                     /site_type="binding"
                     /note="iron (Cys)"
     Site            9
                     /site_type="binding"
                     /note="iron (Cys)"
     Site            39
                     /site_type="binding"
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                     /note="iron (Cys)"
     Site            42
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1: JU0127. Reports rubredoxin - "But...[gi:80482] BLink, Domains, Links

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LOCUS       JU0127                  53 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - 'Butyribacterium methylotrophicum.
ACCESSION   JU0127
VERSION     JU0127  GI:80482
DBSOURCE    pir: locus JU0127;

            summary: #length 53 #molecular-weight 5672 #checksum 6265
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 31-Mar-1990 #sequence_revision 22-Jul-1994 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Butyribacterium methylotrophicum
  ORGANISM  Butyribacterium methylotrophicum
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Saeki,K., Yao,Y., Wakabayashi,S., Shen,G.J., Zeikus,J.G. and
            Matsubara,H.
  TITLE     Ferredoxin and rubredoxin from Butyribacterium methylotrophicum:
            complete primary structures and construction of phylogenetic trees
  JOURNAL   J. Biochem. 106 (4), 656-662 (1989)
  MEDLINE   90110065
   PUBMED   2606914
COMMENT     Rubredoxin is a nonheme iron protein and substitutes for ferredoxin
            in some enzymatic reactions.
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Butyribacterium methylotrophicum"
                     /db_xref="taxon:1487"
     Protein         1..53
                     /product="rubredoxin"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     Region          3..49
                     /region_name="domain"
                     /note="rubredoxin homology #label RUB"
     Site            6
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     Site            39
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Nov 8 2004 13:44:10

1: JU0074. Reports rubredoxin - Clos...[gi:80528] BLink, Domains, Links

```
LOCUS       JU0074                  54 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - Clostridium perfringens.
ACCESSION   JU0074
VERSION     JU0074  GI:80528
DBSOURCE    pir: locus JU0074;

            summary: #length 54 #molecular-weight 6004 #checksum 1502
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 28-Feb-1990 #sequence_revision 22-Jul-1994 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Clostridium perfringens
  ORGANISM  Clostridium perfringens
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 54)
  AUTHORS   Seki,Y., Seki,S., Satoh,M., Ikeda,A. and Ishimoto,M.
  TITLE     Rubredoxin from Clostridium perfringens: complete amino acid
            sequence and participation in nitrate reduction
  JOURNAL   J. Biochem. 106 (2), 336-341 (1989)
  MEDLINE   90036784
   PUBMED   2553684
COMMENT     The protein is reduced with NADH in the presence of a specific
            NAD(P)H oxidoreductase.
FEATURES             Location/Qualifiers
     source          1..54
                     /organism="Clostridium perfringens"
                     /db_xref="taxon:1502"
     Protein         1..54
                     /product="rubredoxin"
     Region          3..49
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                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     Region          3..49
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                     /note="rubredoxin homology #label RUB"
     Site            6
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     Site            39
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Nov 8 2004 13:44:10

1: A33182. Reports rubredoxin - Clos...[gi:98633] BLink, Domains, Links

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LOCUS       A33182                    53 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - Clostridium sticklandii.
ACCESSION   A33182
VERSION     A33182  GI:98633
DBSOURCE    pir: locus A33182;

            summary: #length 53 #molecular-weight 5889 #checksum 6706
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 03-Feb-1994 #sequence_revision 03-Feb-1994 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Clostridium sticklandii
  ORGANISM  Clostridium sticklandii
            Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 53)
  AUTHORS   Meyer,J., Gagnon,J. and Moulis,J.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (??-APR-1991) to the Protein Sequence Database
FEATURES             Location/Qualifiers
     source          1..53
                     /organism="Clostridium sticklandii"
                     /db_xref="taxon:1511"
     Protein         1..53
                     /product="rubredoxin"
     Site            1
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                     /note="N-formylmethionine"
     Region          3..51
                     /region_name="Rubredoxin"
                     /note="rubredoxin"
                     /db_xref="CDD:24175"
     Region          3..49
                     /region_name="domain"
                     /note="rubredoxin homology #label RUB"
     Site            6
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1: JX0241. Reports rubredoxin - Desu...[gi:320090] BLink, Domains, Links

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LOCUS       JX0241                    52 aa            linear   BCT 24-OCT-1997
DEFINITION  rubredoxin - Desulfovibrio vulgaris (strain Miyazaki).
ACCESSION   JX0241
VERSION     JX0241  GI:320090
DBSOURCE    pir: locus JX0241;

            summary: #length 52 #molecular-weight 5598 #checksum 1997
            ;
            superfamily: rubredoxin; rubredoxin homology
            ;
            PIR dates: 10-Jun-1993 #sequence_revision 22-Jul-1994 #text_change
            24-Oct-1997
            .
KEYWORDS    electron transfer; iron; metalloprotein.
SOURCE      Desulfovibrio vulgaris
  ORGANISM  Desulfovibrio vulgaris
            Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales;
            Desulfovibrionaceae; Desulfovibrio.
REFERENCE   1  (residues 1 to 52)
  AUTHORS   Shimizu,F., Ogata,M., Yagi,T., Wakabayashi,S. and Matsubara,H.
  TITLE     Amino acid sequence and function of rubredoxin from Desulfovibrio
            vulgaris Miyazaki
  JOURNAL   Biochimie 71 (11-12), 1171-1177 (1989)
  MEDLINE   90234754
   PUBMED   2561345
FEATURES             Location/Qualifiers
     source          1..52
                     /organism="Desulfovibrio vulgaris"
                     /db_xref="taxon:881"
     Protein         1..52
                     /product="rubredoxin"
     Site            1
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                     /note="N-formylmethionine (partial)"
     Region          3..51
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     Region          3..49
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     Site            6
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     Site            9
                     /site_type="binding"
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1: U21086. Reports Methanobacterium ...[gi:710355] Links

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LOCUS       MTU21086                2843 bp    DNA     linear   BCT 05-MAY-1997
DEFINITION  Methanobacterium thermoautotrophicum flavoprotein (fpaA), (orfX),
            and (rdxA) genes, complete cds.
ACCESSION   U21086
VERSION     U21086.1  GI:710355
KEYWORDS    .
SOURCE      Methanothermobacter thermautotrophicus
  ORGANISM  Methanothermobacter thermautotrophicus
            Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales;
            Methanobacteriaceae; Methanothermobacter.
REFERENCE   1  (bases 1 to 2843)
  AUTHORS   Nolling,J., Ishii,M., Koch,J., Pihl,T.D., Reeve,J.N., Thauer,R.K.
            and Hedderich,R.
  TITLE     Characterization of a 45-kDa flavoprotein and evidence for a
            rubredoxin, two proteins that could participate in electron
            transport from H2 to CO2 in methanogenesis in Methanobacterium
            thermoautotrophicum
  JOURNAL   Eur. J. Biochem. 231 (3), 628-638 (1995)
  MEDLINE   95377292
   PUBMED   7649162
REFERENCE   2  (bases 1 to 2843)
  AUTHORS   Nolling,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-FEB-1995) Jork Nolling, Microbiology, The Ohio State
            University, 484 W. 12th, Ave., Columbus, OH 43210, USA
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="delta H"
                     /db_xref="taxon:145262"
     gene            335..1549
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     CDS             335..1549
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                     /note="uses FMN as cofactor; contains C-terminal located
                     flavodoxin domain; similar to product encoded by
                     Rhodobacter capsulatus nif region orf14, GenBank Accession
                     Number X72888"
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     CDS             1542..1922
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                     proteins"
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                     /product="unknown"
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      181 agaatatcgt gaaattatgt ctcctcttca ttggcccatg ttatgtctga aggactatat
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      481 tgacaactca tatccaggga ccttccagga gctcatggca aggatggagg atgcattcaa
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      601 cagcggagtg ctggtggaac ttcacaggag gttccctgag gcagagatcc actgcacaga
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      901 catactcatg gatgctgcca aaaaattcta tgcgaacctc ataacacccc tatcaaagct
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     1501 tgcatgcttc gaggctggaa gaaggcttgc aggtgatctc aatgaatgaa aacatcaccg
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```
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1741 agctaaacgg tgtgatcagg gaaaacctca gggaaaacct tgagatgatg cttgaggggg
1801 aggaggcggc taacagggag aaacgtgagg cagccctcag ggcaagggat gcggtgttga
1861 tgaggcccat gacttctttg atgagagctc aagggacgag ggaaggcatg ctgccatgct
1921 gaggggactc ctccagaggt acttccaggg ataaccatga agatctgcag aatatgcggc
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2041 ggtgttggga aggaggaact ccaggattcc gccgagcccc tgggggtcg ggaccccctc
2101 atgcttattt tcagggccat gactgtgggg ctctggaggg ttctgggtaa tggatcccag
2161 ggagttacaa gggagatggg gtctgtgatt gcagacaaca tcaggcacgg ggatgatccc
2221 ctgaaatcag ccgcagacta cttcatcgaa catggatttg cagcgtccat cagcacagac
2281 actgaaaact ttgccctcaa tgttaaaaac tgcagtttct atggattctg ttgttcactg
2341 gaggaggatg gggtgctttt atcaacctgc ccctatgcaa acacggccgc tgcagtcctt
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2521 gccaccgaag cccctctgat tcagaaatca gcatagggtc tctccatacc cgcacgcttc
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2701 ctgagggcgt ccacctctgt gaagacctcg ccgggtacag gtaacattcc gggaccctca
2761 gtgccggctg aattcattcg gagcgctgta ctgtaaaggt cgcctggtat catcttttca
2821 agtccaaccg ggactatgag ctc
//
```

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MMDB Structure Summary

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Alzheimer's Disease Amyloid Beta-Peptide (Residues 1 - 28) (E.C. Number Not Assigned) (Nmr, Minimized Average Structure).

Deposition: J.Talafous, K.J.Marcinowski, G.Klopman & M.G.Zagorski, 21-Oct-94

Taxonomy: Homo sapiens

Reference: PubMed **MMDB:** 230 **PDB:** 1AMB

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

NCBI

MMDB Structure Summary

PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

Description: Alzheimer's Disease Amyloid Beta-Peptide (Residues 1 - 28) (E.C. Number Not Assigned) (Nmr, 5 Structures).

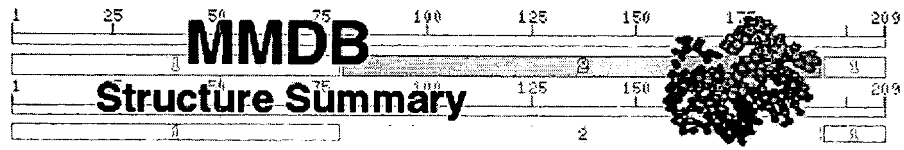
Deposition: J.Talafous, K.J.Marcinowski, G.Klopman & M.G.Zagorski, 14-Nov-94

Taxonomy: Homo sapiens

Reference: PubMed **MMDB:** 231 **PDB:** 1AMC

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.



PubMed BLAST Structure Taxonomy OMIM **Help?** **Cn3d**

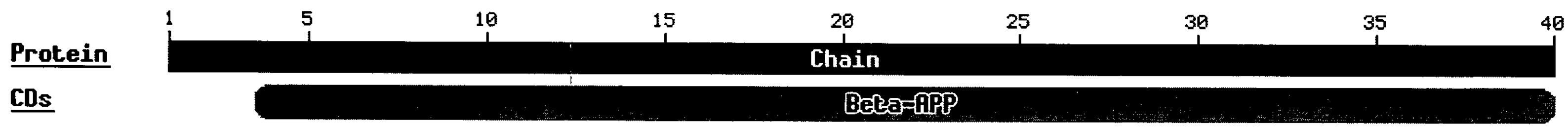
Description: The Solution Structure Of Amyloid Beta-Peptide (1-40) In A Water-Micelle Environment. Is The Membrane-Spanning Domain Where We Think It Is? Nmr, 10 Structures.

Deposition: M.Coles, W.Bicknell, A.A.Watson, D.P.Fairlie & D.J.Craik, 7-Apr-98

Taxonomy: Homo sapiens

Reference: PubMed **MMDB:** 7992 **PDB:** 1BA4

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***



Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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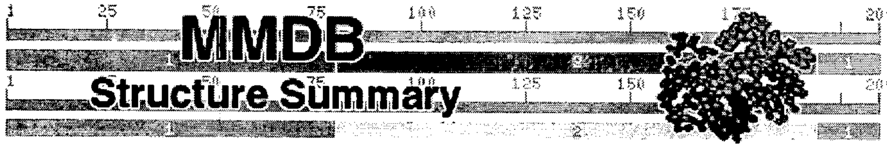


PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

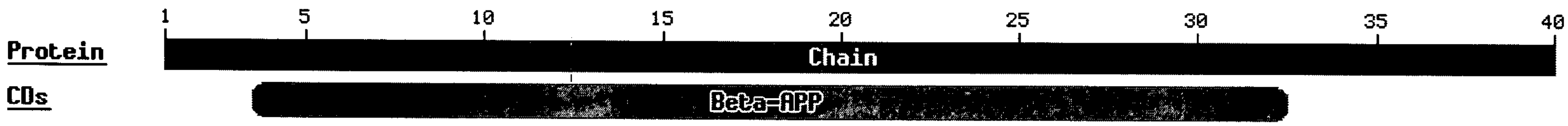
Description: Solution Structure Of The Methionine-Oxidized Amyloid Beta-Peptide (1-40). Does Oxidation Affect Conformational Switching? Nmr, 10 Structures.

Deposition: A.A.Watson, D.P.Fairlie & D.J.Craik, 22-Apr-98

Taxonomy: Homo sapiens

Reference: PubMed **MMDB:** 7993 **PDB:** 1BA6

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***



NCBI MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Solution Nmr Structure Of Amyloid Beta[e16], Residues 1-28, 14 Structures.
Deposition: S.-A.Poulsen, A.A.Watson & D.J.Craik, 23-Jun-98
Taxonomy: Homo sapiens
Reference: PubMed **MMDB:** 8655 **PDB:** 1BJB

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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MMDB Structure Summary

PubMed | BLAST | Structure | Taxonomy | OMIM | **Help?** | **Cn3d**

Description: Solution Nmr Structure Of Amyloid Beta[f16], Residues 1-28, 15 Structures.

Deposition: S.-A.Poulsen, A.A.Watson & D.J.Craik, 23-Jun-98

Taxonomy: Homo sapiens

Reference: PubMed **MMDB:** 9179 **PDB:** 1BJC

View 3D Structure **of** Best Model **with** Cn3D Display NEW ***Get Cn3D 4.1!***

Citing MMDB: Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH, "*MMDB: Entrez's 3D-structure database*", **Nucleic Acids Res. 2003 Jan; 31(1)**: 474-7.

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1: AAA37139. Reports beta-amyloid prot...[gi:309085]

BLink, Domains, Links

```
LOCUS       AAA37139                 695 aa            linear   ROD 11-JUN-1993
DEFINITION  beta-amyloid protein.
ACCESSION   AAA37139
VERSION     AAA37139.1  GI:309085
DBSOURCE    locus MUSABPPA accession M18373.1
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 695)
  AUTHORS   Yamada,T., Sasaki,H., Furuya,H., Miyata,T., Goto,I. and Sakaki,Y.
  TITLE     Complementary DNA for the mouse homolog of the human amyloid beta
            protein precursor
  JOURNAL   Biochem. Biophys. Res. Commun. 149 (2), 665-671 (1987)
  MEDLINE   88106489
   PUBMED   3322280
COMMENT     On Jul 26, 1993 this sequence version replaced gi:191569.
            Draft entry and computer readable copy of sequence [1] kindly
            provided by T.Yamada (17-MAR-1988).
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..695
                     /organism="Mus musculus"
                     /db_xref="taxon:10090"
     Protein         1..695
                     /name="beta-amyloid protein"
     Region          24..188
                     /region_name="amyloid A4"
                     /note="A4_EXTRA"
                     /db_xref="CDD:22659"
     Region          <309..505
                     /region_name="ATPase involved in DNA repair [DNA
                     replication, recombination, and repair]"
                     /note="SbcC"
                     /db_xref="CDD:10293"
     Region          600..>630
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
     CDS             1..695
                     /coded_by="M18373.1:139..2226"
ORIGIN
        1 mlpslallll aawtvralev ptdgnaglla epqiamfcgk lnmhmnvqng kwesdpsgtk
       61 tcigtkegil qycqevypel qitnvveanq pvtiqnwckr grkqckthth ivipyrclvg
      121 efvsdallvp dkckflhqer mdvcethlhw htvaketcse kstnlhdygm llpcgidkfr
      181 gvefvccpla eesdsvdsad aeeddsdvww vgadtdyadg gedkvvevae eeevadveee
      241 eadddedved gdeveeeaee pyeeatertt statttttt esveevvrvp ttaastpdav
```

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301 dkyletpgde nehahfqkak erleakhrer msqvmrewee aerqaknlpk adkkaviqhf
361 qekvesleqe aanerqqlve thmarveaml ndrrrldlen yiialqavpp rphhvfnmlk
421 kyvraeqkdr qhtlkhfehv rmvdpkkatq irsqvmthlr viyermnqsl sllynvpava
481 eeiqdevdel lqkeqnysdd vlanmisepr isygndalmp sltetkttve llpvngefsl
541 ddlqpwhpfg vdsvpanten evepvdarpa adrglttrpg sgltniktee isevkmdaef
601 ghdsgfevrh qklvffaedv gsnkgaiigl mvggvviatv ivitlvmlkk kqytsihhgv
661 vevdaavtpe erhlskmqqn gyenptykff eqmqn
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1: AAA51726. Reports beta-amyloid A4...[gi:178573]

BLink, Domains, Links

```
LOCUS       AAA51726                 264 aa            linear   PRI 31-OCT-1994
DEFINITION  beta-amyloid A4.
ACCESSION   AAA51726
VERSION     AAA51726.1  GI:178573
DBSOURCE    locus HUMAMY4A accession M18734.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 264)
  AUTHORS   Zain,S.B., Salim,M., Chou,W.G., Sajdel-Sulkowska,E.M., Majocha,R.E.
            and Marotta,C.A.
  TITLE     Molecular cloning of amyloid cDNA derived from mRNA of the
            Alzheimer disease brain: coding and noncoding regions of the fetal
            precursor mRNA are expressed in the cortex
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 85 (3), 929-933 (1988)
  MEDLINE   88124954
   PUBMED   2893379
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..264
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
                     /map="21q21.2"
     Protein         1..264
                     /name="beta-amyloid A4"
     Region          169..>199
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
     CDS             1..264
                     /gene="APP"
                     /coded_by="M18734.1:<1..797"
                     /codon_start=2
                     /db_xref="GDB:G00-119-692"
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       61 qkeqnysddv lanmisepri sygndalmps ltetkttvel lpvngefsld dlqpwhsfga
      121 dsvpantene vepvdarpaa drglttrpgs gltnikteei sevkmdaefr hdsgyevhhq
      181 klvffaedvg snkgaiiglm vggvviatvi vitlvmlkkk qytsihhgvv evdaavtpee
      241 rhlskmqqng yenptykffe qmqn
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1: AAA71550. Reports Sequence 1 from p...[gi:912096] BLink, Links

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LOCUS       AAA71550                 40 aa            linear   PAT 26-JUL-1995
DEFINITION  Sequence 1 from patent US 5434050.
ACCESSION   AAA71550
VERSION     AAA71550.1  GI:912096
DBSOURCE    accession AAA71550.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 40)
  AUTHORS   Maggio,J.E. and Mantyh,P.W.
  TITLE     Labelled .beta.-amyloid peptide and methods of screening for
            Alzheimer's disease
  JOURNAL   Patent: US 5434050-A 1 18-JUL-1995;
FEATURES             Location/Qualifiers
     source          1..40
                     /organism="unknown"
     Region          4..40
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
ORIGIN
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1: AAA71551. Reports Sequence 2 from p...[gi:912097] BLink, Links

```
LOCUS       AAA71551                  42 aa            linear   PAT 26-JUL-1995
DEFINITION  Sequence 2 from patent US 5434050.
ACCESSION   AAA71551
VERSION     AAA71551.1  GI:912097
DBSOURCE    accession AAA71551.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 42)
  AUTHORS   Maggio,J.E. and Mantyh,P.W.
  TITLE     Labelled .beta.-amyloid peptide and methods of screening for
            Alzheimer's disease
  JOURNAL   Patent: US 5434050-A 2 18-JUL-1995;
FEATURES             Location/Qualifiers
     source          1..42
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     Region          4..42
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                     /note="Beta-APP"
                     /db_xref="CDD:5537"
ORIGIN
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//
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1: AAA75654. Reports Sequence 1 from p...[gi:997353] BLink, Links

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LOCUS       AAA75654                  16 aa            linear   PAT 26-SEP-1995
DEFINITION  Sequence 1 from patent US 5441870.
ACCESSION   AAA75654
VERSION     AAA75654.1  GI:997353
DBSOURCE    accession AAA75654.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 16)
  AUTHORS   Seubert,P.A., Schenk,D.B. and Fritz,L.C.
  TITLE     Methods for monitoring cellular processing of .beta.-amyloid
            precursor protein
  JOURNAL   Patent: US 5441870-A 1 15-AUG-1995;
FEATURES             Location/Qualifiers
     source          1..16
                     /organism="unknown"
ORIGIN
        1 gsgltnikte eisevk
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☐1: AAB13827. Reports Sequence 8 from p...[gi:1610848] BLink, Links

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LOCUS       AAB13827                 770 aa            linear   PAT 07-OCT-1996
DEFINITION  Sequence 8 from patent US 5525714.
ACCESSION   AAB13827
VERSION     AAB13827.1  GI:1610848
DBSOURCE    accession AAB13827.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 770)
  AUTHORS   Van Broeckhoven,C., Martin,J.-J., Hendriks,L. and Cras,P.
  TITLE     Mutated form of the .beta.-amyloid precursor protein gene
  JOURNAL   Patent: US 5525714-A 8 11-JUN-1996;
FEATURES             Location/Qualifiers
     source          1..770
                     /organism="unknown"
     Region          24..188
                     /region_name="amyloid A4"
                     /note="A4_EXTRA"
                     /db_xref="CDD:22659"
     Region          291..342
                     /region_name="BPTI/Kunitz family of serine protease
                     inhibitors"
                     /note="KU"
                     /db_xref="CDD:5332"
     Region          <384..580
                     /region_name="ATPase involved in DNA repair [DNA
                     replication, recombination, and repair]"
                     /note="SbcC"
                     /db_xref="CDD:10293"
     Region          675..>705
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
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       61 tcidtkegil qycqevypel qitnvveanq pvtiqnwckr grkqckthph fvipyrclvg
      121 efvsdallvp dkckflhqer mdvcethlhw htvaketcse kstnlhdygm llpcgidkfr
      181 gvefvccpla eesdnvdsad aeeddsdvww ggadtdyadg sedkvvevae eeevaeveee
      241 eadddedded gdeveeeaee pyeeatertt siatttttt esveevvrev cseqaetgpc
      301 ramisrwyfd vtegkcapff yggcggnrnn fdteeycmav cgsamsqsll kttqeplard
      361 pvklpttaas tpdavdkyle tpgdenehah fqkakerlea khrermsqvm reweeaerqa
      421 knlpkadkka viqhfqekve sleqeaaner qqlvethmar veamlndrrr lalenyital
      481 qavpprprhv fnmlkkyvra eqkdrqhtlk hfehvrmvdp kkaaqirsqv mthlrviyer
      541 mnqslsllyn vpavaeeiqd evdellqkeq nysddvlanm iseprisygn dalmpsltet
      601 kttvellpvn gefslddlqp whsfgadsvp antenevepv darpaadrgl ttrpgsgltn
      661 ikteeisevk mdaefrhdsg yevhhqklvf faedvgsnkg aiiglmvggv viatvivitl
      721 vmlkkkqyts ihhgvvevda avtpeerhls kmqqngyenp tykffeqmqn
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1: AAB15507. Reports Sequence 1 from p...[gi:1612527] BLink, Links

```
LOCUS       AAB15507                  43 aa            linear   PAT 07-OCT-1996
DEFINITION  Sequence 1 from patent US 5552426.
ACCESSION   AAB15507
VERSION     AAB15507.1  GI:1612527
DBSOURCE    accession AAB15507.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 43)
  AUTHORS   Lunn,W.H.W., Monn,J.A. and Zimmerman,D.M.
  TITLE     Methods for treating a physiological disorder associated with
            .beta.-amyloid peptide
  JOURNAL   Patent: US 5552426-A 1 03-SEP-1996;
FEATURES             Location/Qualifiers
     source          1..43
                     /organism="unknown"
ORIGIN
        1 daefrhdsgy evhhqklvff aedvgsnkga iiglmvggvv iat
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1: AAB15508. Reports Sequence 2 from p...[gi:1612528] BLink, Links

```
LOCUS       AAB15508                  40 aa            linear   PAT 07-OCT-1996
DEFINITION  Sequence 2 from patent US 5552426.
ACCESSION   AAB15508
VERSION     AAB15508.1  GI:1612528
DBSOURCE    accession AAB15508.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 40)
  AUTHORS   Lunn,W.H.W., Monn,J.A. and Zimmerman,D.M.
  TITLE     Methods for treating a physiological disorder associated with
            .beta.-amyloid peptide
  JOURNAL   Patent: US 5552426-A 2 03-SEP-1996;
FEATURES             Location/Qualifiers
     source          1..40
                     /organism="unknown"
     Region          4..40
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
ORIGIN
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1: AAB19991. Reports amyloid precursor...[gi:236721] BLink, Domains, Links

```
LOCUS       AAB19991                  49 aa            linear   PRI 07-MAY-1993
DEFINITION  amyloid precursor protein; APP [Homo sapiens].
ACCESSION   AAB19991
VERSION     AAB19991.1  GI:236721
DBSOURCE    locus S57665 accession S57665.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 49)
  AUTHORS   Murrell,J., Farlow,M., Ghetti,B. and Benson,M.D.
  TITLE     A mutation in the amyloid precursor protein associated with
            hereditary Alzheimer's disease
  JOURNAL   Science 254 (5028), 97-99 (1991)
  MEDLINE   92022553
   PUBMED   1925564
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 57667] from the original journal article.
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..49
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
     Protein         1..49
                     /product="amyloid precursor protein"
                     /note="transmembrane domain; APP"
     Region          <1..25
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
     CDS             1..49
                     /gene="amyloid precursor protein, APP"
                     /coded_by="S57665.1:109..255"
ORIGIN
        1 vffaedvgsn kgaiiglmvg gvviatvifi tlvmlkkkqy tsihhgvve
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1: AAB23544. Reports beta-amyloid pept...[gi:257056]

BLink, Domains, Links

```
LOCUS       AAB23544                  33 aa            linear   PRI 08-MAY-1993
DEFINITION  beta-amyloid peptide, A beta {N-terminal} [human, cerebrospinal
            fluid, conditioned medium of mixed-brain cell cultures, Peptide
            Partial, 33 aa].
ACCESSION   AAB23544
VERSION     AAB23544.1  GI:257056
DBSOURCE    accession AAB23544.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 33)
  AUTHORS   Seubert,P., Vigo-Pelfrey,C., Esch,F., Lee,M., Dovey,H., Davis,D.,
            Sinha,S., Schlossmacher,M., Whaley,J., Swindlehurst,C. et,al.
  TITLE     Isolation and quantification of soluble Alzheimer's beta-peptide
            from biological fluids
  JOURNAL   Nature 359 (6393), 325-327 (1992)
  MEDLINE   93024877
   PUBMED   1406936
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 114715] from the original journal article.
COMMENT     Method: sequenced peptide, ordered by homology.
FEATURES             Location/Qualifiers
     source          1..33
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
     Protein         1..33
                     /product="beta-amyloid peptide"
                     /note="A beta"
     Region          4..>33
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
ORIGIN
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1: AAB28602. Reports beta-amyloid pept...[gi:435822] BLink, Domains, Links

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LOCUS       AAB28602                  28 aa            linear   PRI 18-DEC-1993
DEFINITION  beta-amyloid peptide, A beta {N-terminal} [human, patient with
            Alzheimer's disease, cerebrospinal fluid, Peptide Partial, 28 aa].
ACCESSION   AAB28602
VERSION     AAB28602.1  GI:435822
DBSOURCE    accession AAB28602.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 28)
  AUTHORS   Vigo-Pelfrey,C., Lee,D., Keim,P., Lieberburg,I. and Schenk,D.B.
  TITLE     Characterization of beta-amyloid peptide from human cerebrospinal
            fluid
  JOURNAL   J. Neurochem. 61 (5), 1965-1968 (1993)
  MEDLINE   94045685
   PUBMED   8229004
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 139006] from the original journal article.
COMMENT     Method: direct peptide sequencing.
FEATURES             Location/Qualifiers
     source          1..28
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
     Protein         1..28
                     /product="beta-amyloid peptide"
                     /note="A beta"
     Region          4..>27
                     /region_name="Beta-amyloid peptide (beta-APP)"
                     /note="Beta-APP"
                     /db_xref="CDD:5537"
ORIGIN
        1 daefrhdsgy evhhqklvff aedvgsnx
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1: AAB29001. Reports beta-amyloid-(1-4...[gi:455742] BLink, Links

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LOCUS       AAB29001                  19 aa            linear   PRI 18-FEB-1994
DEFINITION  beta-amyloid-(1-42), A beta-(1-42)=amyloid major component
            {N-terminal} [human, leptomeningeal blood vessels, Peptide Partial,
            19 aa].
ACCESSION   AAB29001
VERSION     AAB29001.1  GI:455742
DBSOURCE    accession AAB29001.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 19)
  AUTHORS   Roher,A.E., Lowenson,J.D., Clarke,S., Woods,A.S., Cotter,R.J.,
            Gowing,E. and Ball,M.J.
  TITLE     beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid
            deposits: implications for the pathology of Alzheimer disease
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 90 (22), 10836-10840 (1993)
  MEDLINE   94068497
   PUBMED   8248178
  REMARK    GenBank staff at the National Library of Medicine created this
            entry [NCBI gibbsq 141019] from the original journal article.
COMMENT     Method: direct peptide sequencing.
FEATURES             Location/Qualifiers
     source          1..19
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
     Protein         1..19
                     /product="beta-amyloid-(1-42)"
                     /name="amyloid major component"
                     /note="A beta-(1-42)"
ORIGIN
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1: AAB56103. Reports Sequence 1 from p...[gi:2097271] BLink, Links

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LOCUS       AAB56103                  16 aa            linear   PAT 14-MAY-1997
DEFINITION  Sequence 1 from patent US 5605811.
ACCESSION   AAB56103
VERSION     AAB56103.1  GI:2097271
DBSOURCE    accession AAB56103.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 16)
  AUTHORS   Seubert,P.A., Schenk,D.B. and Fritz,L.C.
  TITLE     Methods and compositions for monitoring cellular processing of
            beta-amyloid precursor protein
  JOURNAL   Patent: US 5605811-A 1 25-FEB-1997;
FEATURES             Location/Qualifiers
     source          1..16
                     /organism="unknown"
ORIGIN
        1 gsgltnikte eisevk
//
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Nov 8 2004 13:44:10

1: AAB56104. Reports Sequence 2 from p...[gi:2097272] BLink, Links

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LOCUS       AAB56104                  17 aa            linear   PAT 14-MAY-1997
DEFINITION  Sequence 2 from patent US 5605811.
ACCESSION   AAB56104
VERSION     AAB56104.1  GI:2097272
DBSOURCE    accession AAB56104.1
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (residues 1 to 17)
  AUTHORS   Seubert,P.A., Schenk,D.B. and Fritz,L.C.
  TITLE     Methods and compositions for monitoring cellular processing of
            beta-amyloid precursor protein
  JOURNAL   Patent: US 5605811-A 2 25-FEB-1997;
FEATURES             Location/Qualifiers
     source          1..17
                     /organism="unknown"
ORIGIN
        1 ysgltnikte eisevkm
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JOURNAL OF BACTERIOLOGY, Sept. 1989, p. 4996–5004
0021-9193/89/094996-09$02.00/0

Vol. 171, No. 9

Analysis of the Transcriptional Unit Encoding the Genes for Rubredoxin (*rub*) and a Putative Rubredoxin Oxidoreductase (*rbo*) in *Desulfovibrio vulgaris* Hildenborough

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The nucleotide sequence of a 2.0-kilobase-pair *Eco*RI restriction fragment upstream from the gene (*rub*, 162 base pairs) encoding rubredoxin from *Desulfovibrio vulgaris* Hildenborough indicates that it is part of a larger transcriptional unit, containing an additional 378-base-pair open reading frame which terminates 16 nucleotides from the translational start of the *rub* gene and could encode a polypeptide of 14 kilodaltons (kDa). Northern (RNA) blotting of RNA isolated from both *D. vulgaris* Hildenborough and *Escherichia coli* TG2 transformed with plasmid pJK29, which contains both genes on a 1.1-kilobase-pair *Sal*I insert, confirms that the genes for this 14-kDa polypeptide and rubredoxin are present on a single transcript of 680 nucleotides. Strong evidence that the 14-kDa polypeptide is also a redox protein is provided by the fact that its NH_2 terminus is homologous to desulforedoxin, which has been isolated from *D. gigas* as a small dimeric redox protein (36 amino acids per monomer), coordinating two iron atoms. Since rubredoxin is a potential redox partner for the 14-kDa protein, it has been tentatively named rubredoxin oxidoreductase, produced by the *rbo* gene. Southern blotting indicates that the *rbo-rub* operon is present in several species and strains of sulfate-reducing bacteria.

Rubredoxins are small, electron-carrying proteins (molecular mass, 6 kilodaltons [kDa]), which are found in the cytoplasm of several anaerobic bacteria, e.g., *Desulfovibrio gigas* (8), *D. vulgaris* Hildenborough (7), *D. desulfuricans* (15), *Thermodesulfobacterium commune* (24), *Clostridium pasteurianum* (36), *Peptostreptococcus elsdenii* (4), and *Peptococcus aerogenes* (3). The amino acid sequences of these rubredoxins have been determined (3, 4, 7, 8, 15, 36), and the three-dimensional structures of rubredoxins from *C. pasteurianum*, *D. vulgaris*, *D. gigas*, and *D. desulfuricans*, which has a very low molecular mass (only 5.2 kDa) have been solved (2, 13, 14, 32, 41–43). The redox center of rubredoxin consists of a single iron atom (redox potential, −50 to 0 mV), coordinated to four cysteinyl sulfurs. This redox potential is relatively high, since dissimilatory sulfate reduction by *Desulfovibrio* species requires electrons at −400 to −200 mV (17, 18, 23, 27), and it is not clear, therefore, which electron transfer reaction is catalyzed by rubredoxin. Several attempts have been made to define the redox partners of rubredoxin by biochemical experiments. By using the rubredoxin-mediated reduction of eucaryotic cytochrome *c* by NADH as an assay, an NADH-rubredoxin oxidoreductase was (partially) purified from *D. gigas* (16) and *C. acetobutylicum* (25). The *Clostridium* enzyme was found to have flavin adenine dinucleotide as the prosthetic group and to consist of a single subunit with a molecular mass of 41 kDa (25). The *D. gigas* enzyme shows specificity for rubredoxin from *D. gigas* ($K_m = 6.2 \times 10^{-6}$ M) relative to rubredoxins from *D. vulgaris* ($K_m = 5.3 \times 10^{-5}$ M) and *C. pasteurianum* ($K_m = 1.0 \times 10^{-4}$ M), which show 71 and 63% sequence identity, respectively, with *D. gigas* rubredoxin. The subunit molecular mass and nature of the cofactor have not been reported for the *D. gigas* enzyme, and it has not been isolated from *D. vulgaris*.

A different approach to the identification of possible redox partners for rubredoxin, which makes use of the fact that the gene encoding rubredoxin (*rub*) from *D. vulgaris* Hildenborough was recently cloned from a λ library of the *D. vulgaris* chromosome and its nucleotide sequence was determined (37), is taken here. No typical promoter consensus sequence (29) was found in the region immediately upstream from the *rub* gene, which instead appears to contain the 3′ end of another reading frame, indicating that the *rub* gene may be part of a larger transcriptional unit. Since at least one gene encoding a redox protein interacting with rubredoxin could be present on this larger transcript, the region upstream from the *rub* gene is characterized in detail in the present paper.

MATERIALS AND METHODS

Strains, vectors, and media. The bacterial strains, plasmids, and cloning vectors used in this work are described in Table 1. For the isolation of RNA, *D. vulgaris* Hildenborough was grown anaerobically in a medium made up from the following solutions, described by Pfennig et al. (26): solution 1 (1 liter, kept under 90% N_2–10% CO_2), solution 2 (1 ml), solution 3 (1 ml), solution 4 (30 ml), solution 5 (3 ml), 15% (wt/vol) of sodium lactate (10 ml), solution 8 (1 ml), solution 9 (0.1 ml), and Wolin vitamins (10 ml) (44). This medium allows a high growth rate of *D. vulgaris*, and RNA can successfully be isolated from cells grown for 16 h at 30°C at a 5% (vol/vol) inoculum. Chromosomal DNA, isolated from 15 different species and strains of sulfate-reducing bacteria (see Table 2) after growth on Postgate medium C (27), was a gift from Helen Kent, AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, England. DNA from *D. vulgaris* Miyazaki was a gift from T. Yagi, Department of Chemistry, Shizuoka University, Shizuoka, Japan, and DNA from *D. salexigens* NCIMB 8365 was donated by D. W. S. Westlake, Department of Microbiology, University of Alberta, Edmonton, Canada. *Escherichia coli* TG2 was grown in TY medium (22) containing 10 g of tryptone, 5

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TABLE 1. Bacterial strains and DNA vectors used in this study

Strain or vector	Genotype, phenotype, comments, reference
Strains	
D. vulgaris subsp. *vulgaris* Hildenborough[a]	NCIMB 8303; isolated from clay soil near Hildenborough, U.K. (27)
E. coli TG2[b]	Δ(*lac-pro*) *supE thi hsdM hsdR recA* F' (*traD36 proAB*$^+$ *lacZ*ΔM15*I*^{q}); from T. J. Gibson.
Vectors	
pUC8, pUC9	Amp^r (35)
pJK15	This study (Fig. 1)
pJK29	This study (Fig. 1)
pRbo1	This study (Fig. 1)
pRub1	This study (Fig. 1)

[a] Abbreviated as *D. vulgaris* Hildenborough. A bacteriophage λ gene library has been constructed for this organism (37).
[b] Constructed from *E. coli* JM101 [Δ(*lac-pro*) *supE thi* F' (*traD36proAB*$^+$ *lacZ*ΔM15 *I*^{q}] by T. J. Gibson and M. D. Biggin, Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge, U.K.

g of yeast extract, and 5 g of NaCl per liter of water at pH 7.4.

Biochemical reagents. All enzymes were obtained from Pharmacia, Inc., with the exception of calf alkaline phosphatase, which was from Boehringer Mannheim Biochemicals. The radioisotopes [α-^{35}S]dATP (400 Ci/mmol; 10 mCi/ml), [α-^{32}P]dATP (3,000 Ci/mmol; 10 mCi/ml), and [γ-^{32}P]ATP (3,000 Ci/mmol; 10 mCi/ml) were purchased from Amersham Corp. and were used for dideoxynucleotide sequencing, nick translation, and 3' and 5' end labeling, respectively. Ficoll 400 was purchased from Pharmacia, Inc. Polyvinylpyrrolidone (molecular weight 40,000), bovine serum albumin (fraction V), molecular biology grade sodium dodecyl sulfate (SDS), bakers' yeast tRNA, salmon sperm DNA (sodium salt), and dextran sulfate (molecular weight 500,000) were purchased from Sigma Chemical Co. Low- and high-gelling-temperature (LGT and HGT) agarose were obtained from Bethesda Research Laboratories, Inc. Nitrocellulose and Hybond-N hybridization transfer membranes were obtained from Schleicher & Schuell, Inc., and Amersham, respectively. All other reagent grade chemicals were purchased from either Sigma or Fisher Scientific Co.

DNA cloning. Several recombinant bacteriophages carrying the *rub* gene were isolated from a λ library, as described previously (37). These clones cover 35 kilobase pairs (kb) of the *D. vulgaris* Hildenborough chromosome, and an *Eco*RI restriction map of a part of this region is shown in Fig. 1. A 2.0-kb *Eco*RI fragment and a 1.1-kb *Sal*I fragment (Fig. 1) were cloned into the *Eco*RI and *Sal*I sites of pUC8 (35), generating plasmids pJK15 and pJK29, respectively, which were purified as described elsewhere (40).

Shotgun nucleotide sequencing. The procedure followed for shotgun nucleotide sequencing was essentially that outlined by Bankier and Barrell (5). Plasmid pJK15 was sonicated, and the resulting fragments were end repaired and size fractionated by gel electrophoresis on 1% (wt/vol) LGT agarose. The 400- to 1,000-base-pair (bp) fraction was excised and isolated from the gel and ligated to the replicative form of M13mp8 (21), digested with *Sma*I and calf alkaline phosphatase (5). After being transfected into competent, $CaCl_2$-treated *E. coli* TG2 cells, the ligation mixtures were spread onto TY plates with top agar, containing isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (5). Single-stranded DNA was isolated from white recombinant phage plaques by using a 1.5-ml minipreparation procedure (5). The purified DNAs (2 μl) were spotted on a nitrocellulose filter, which was subsequently baked at 80°C for 1 h under vacuum. The 2.0-kb *Eco*RI insert from pJK15 was isolated by LGT agarose gel electrophoresis, radiolabeled by nick translation

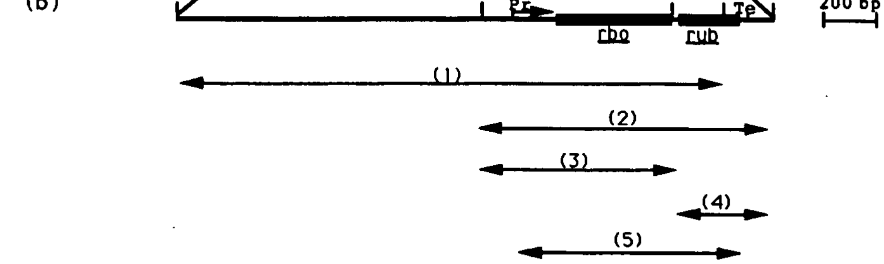

FIG. 1. Survey of DNA containing the *rub* gene of *D. vulgaris* Hildenborough. (a) Location of *Eco*RI (E) restriction sites as determined earlier (37). (b) Map of a 2,234-bp *Eco*RI-*Sal*I region of DNA for which the nucleic acid sequence was determined. The location of restriction sites for *Eco*RI, *Sal*I (S), and *Ava*I (A), the coding regions for the genes encoding rubredoxin (*rub*) and the putative rubredoxin oxidoreductase (*rbo*), and the location of promoter (Pr) and transcription terminator (Te) sequences, are indicated. The following plasmids (inserts) were constructed: 1, pJK15 (2.0 kb EE); 2, pJK29 (1.1 kb SS); 3, pRbo1 (746 bp AS) and 4, pRub1 (347 bp AS). The position of the nucleic acid sequence shown in Fig. 3 is indicated in region 5.

(20), and denatured by boiling. The filter was prehybridized in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.2])–10× Denhardt solution (20)–0.5% (wt/vol) SDS for 15 min at 68°C. The 2.0-kb DNA probe was then added to the prehybridization solution, and hybridization was continued for 16 h at 68°C. After hybridization, the filter was washed in 6× SSC–0.5% (wt/vol) SDS at 68°C for 1 h. The filter was then dried and wrapped in Saran wrap, and positive clones were identified by autoradiography. These were then sequenced by the dideoxy-chain termination procedure developed by Sanger et al. (31), as detailed by Bankier and Barrell (5). The sequencing data were processed and analyzed by using the programs of Staden and McLachlan (33, 34).

Construction of gene-specific probes. The completed nucleotide sequence indicated the presence of a unique *Ava*I site in the 19-bp region separating the *rbo* and *rub* genes (Fig. 1; see also Fig. 3). Plasmid pJK29 was digested with *Ava*I, and the digested DNA was end repaired with the Klenow fragment of DNA polymerase I to generate blunt ends (20). Following inactivation of the polymerase at 68°C, pJK29 was further digested with *Sal*I, resulting in the excision of both the *rbo* and *rub* genes on 746- and 347-bp fragments of DNA, respectively; these were isolated by electrophoresis on 1.5% (wt/vol) LGT agarose. The purified fragments were ligated into pUC8, previously digested with *Sal*I and *Sma*I, to give plasmids pRbo1 and pRub1, respectively. The insert of pRbo1 was gel isolated following digestion with *Hin*dIII and *Eco*RI as a 767-bp *rbo* gene-specific probe, whereas the presence of an *Eco*RI site near the 3′ end of the *rub* gene (Fig. 1; see also Fig. 3) allowed the isolation of a 162-bp *rub* gene-specific probe by digestion of pRub1 with *Eco*RI and gel electrophoresis.

RNA isolation and Northern blotting. RNA was isolated from cultures of *D. vulgaris* Hildenborough and *E. coli* TG2 by the hot-phenol extraction method (11) and stored as the ethanol precipitate at −20°C. Samples of RNA were collected by centrifugation, washed with 70% (vol/vol) ethanol, dried under vacuum, and redissolved in 20 μl of loading buffer, prepared as described elsewhere (10). For Northern (RNA) blotting, RNA samples were electrophoresed on gels containing 1.0% (wt/vol) HGT agarose in 1× MOPS buffer (10× MOPS buffer is 0.2 M 3-*N*-morpholinopropanesulfonic acid, 10 mM EDTA, and 50 mM sodium acetate [pH 7.0]) containing 2% (vol/vol) formaldehyde and then blotted onto a Hybond-N hybridization transfer membrane. Following transfer, the membrane was exposed to shortwave UV light to cross-link the RNA to the filter. Blots were prehybridized for 4 h at 42°C in a solution containing 0.75 M NaCl, 0.075 M sodium citrate (pH 7.0), 50% (vol/vol) formamide, 1% (wt/vol) SDS, 0.1% (wt/vol) Ficoll 400, 0.1% (wt/vol) polyvinylpyrrolidone, 0.1% (wt/vol) bovine serum albumin, and 0.16 mg of boiled bakers' yeast tRNA per ml. Following prehybridization, the blots were hybridized at 42°C for 16 h in the solution described above, also containing 10% (wt/vol) dextran sulfate as a hybridization enhancer, with a nick-translated probe derived from either plasmid pRbo1 or pRub1. Following hybridization, the blots were washed twice for 5 min at room temperature with 100 ml of 0.3 M NaCl–0.06 M Tris hydrochloride (pH 8.0)–0.002 M EDTA, then twice for 15 min at 60°C with 100 ml of the same solution with 0.5% (wt/vol) of SDS added, and, finally, twice for 15 min at room temperature with 0.003 M Tris base, after which the filters were dried and autoradiographed.

S1 nuclease mapping. A probe covering the region upstream from the *rbo* gene was prepared by digestion of pJK29 with *Eco*RI and calf alkaline phosphatase and gel isolation of the 913-bp *Eco*RI fragment, containing the relevant *Sal*I-*Eco*RI fragment. The probe was 5′ end labeled by adding T4 polynucleotide kinase (1 μl; 10 U/μl) and [γ-^{32}P]ATP (2.5 μl; 10 μCi/μl) in the presence of 50 mM Tris hydrochloride (pH 7.6)–10 mM $MgCl_2$–3 mM dithiothreitol–0.1 mM EDTA in a total volume of 10 μl at room temperature for 1 h. The labeled probe was phenol extracted, ethanol precipitated, and dissolved in 20 μl of 80% (vol/vol) formamide–20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.5)–400 mM NaCl (hybridization buffer). RNA (100 μg) was dissolved in 40 μl of hybridization buffer and incubated with the probe at 44°C for 12 h. Following the addition of 240 μl of S1 nuclease buffer (300 mM NaCl, 18 mM sodium acetate [pH 4.6], 10 mM $ZnSO_4$) and 2 μl of S1 nuclease (384,000 U/μl), the hybridization solution was incubated at room temperature for 30 min. The reaction was terminated by the addition of 10 μl of denatured bakers' yeast tRNA (1 μg/μl), and then phenol extraction and ethanol precipitation of the DNA-RNA hybrids for 1 h at −70°C were carried out. The vacuum-dried samples were dissolved in 10 μl of formamide dye mixture, prepared as described by Bankier and Barrell (5), and heated at 80°C for 5 min before being loaded on either a 4 or 6% (wt/vol) denaturing acrylamide gel (5), which was run at 39 W for 2 to 3 h. A partial *Hin*fI digest of plasmid pUC8, labeled by filling the ends with Klenow polymerase in the presence of dGTP, dCTP, dTTP, and [α-^{32}P]dATP, was used as a molecular size marker (1,613, 913, 517, 396, 140, 75, and 65 nucleotides).

Southern blotting. Samples of genomic DNA (3 to 10 μg) from 17 different species and strains of sulfate-reducing bacteria were digested with *Eco*RI, electrophoresed on a 0.7% (wt/vol) HGT agarose gel as described elsewhere (40), and blotted on Hybond-N hybridization transfer membrane. The DNA was bound to the membrane by UV cross-linking as described above, and the blots were prehybridized for 1 h at 42°C with a solution (pH 7.4) containing 50% (vol/vol) formamide, 1 M NaCl, 0.1% (wt/vol) sodium PP_i, 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) Ficoll 400, 10% (wt/vol) dextran sulfate, and 0.175 mg of denatured salmon sperm DNA per ml. A nick-translated pRub1 probe (20) was then added, and hybridization was continued for 16 h. The Southern blots were washed similarly to the Northern blots, dried, and autoradiographed. The pRub1 probe was removed by washing with 0.5 M NaOH–1.5 M NaCl for 30 min and neutralizing for 30 min with 1 M Tris hydrochloride (pH 8.0)–1.5 M NaCl. Removal of the probe was confirmed by autoradiography, after which the blots were rehybridized with the nick-translated pRbo1 probe.

RESULTS

Nucleotide sequence upstream from *rub*. The nucleotide sequence of the 2.0-kb *Eco*RI fragment, containing the region upstream from the *rub* gene, was determined by the random cloning and dideoxy sequencing procedure of Bankier and Barrell (5), and these data were combined with the sequence of the *rub* gene (37) to obtain a sequence of 2,234 nucleotides (nt) extending from an *Eco*RI to a *Sal*I site (Fig. 1b). An identification of possible coding regions with the aid of the codon probability method of Staden and McLachlan (34) in this 2.2-kb sequence is shown in Fig. 2. Apart from the *rub* gene, this analysis indicates the presence of two other plausible genes in frames a and b. The amino acid sequences derived from these possible genes were compared

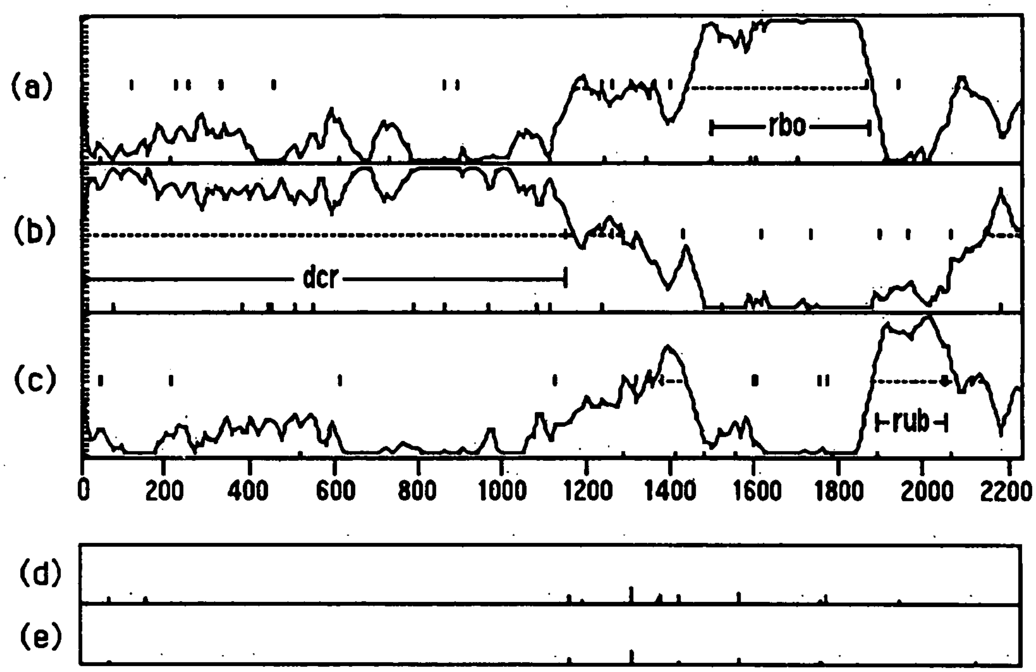

FIG. 2. Statistical analysis of the 2,234-bp region outlined in Fig. 1b. (a to c) The probability that a stretch of sequence (25 bases) is coding is calculated and plotted for each of the three reading frames a, b, and c (36). A codon usage table, which includes all codons of structural genes from *D. vulgaris* Hildenborough, was used as the standard in the calculation. Methionine (ATG) start codons are indicated on the base line, and stop codons are indicated at the half level of each frame. The coding regions for a putative rubredoxin oxidoreductase (*rbo*), a putative *D. vulgaris* chemoreceptor (*dcr*), and rubredoxin (*rub*) are indicated by the calculation as shown in frames a to c, respectively. (d and e) Search for *E. coli* promoters. Sequences with homology to the consensus −35 (TTGACA) and −10 (TATAAT) *E. coli* promoter sequence are indicated in frames d and e, respectively. The scale is in base pairs.

with sequences in the Bionet data base, which indicated unique and extensive homologies with, respectively, desulforedoxin of *D. gigas* (9) and the aspartate and serine chemoreceptors of *Salmonella typhimurium* (30) and *E. coli* (6), respectively. The amino acid sequence that can be derived from the sequenced portion of the *dcr* gene (nt 1 to 1158) lacks approximately 140 NH_2-terminal amino acids, and further nucleic acid sequencing is required for the completion of the sequence of the putative *Desulfovibrio* chemoreceptor protein. The homology of the *dcr* gene product with these two known chemotactically active proteins will therefore not be further discussed here, allowing a focus on the gene immediately preceding the *rub* gene. In view of the homology with desulforedoxin, as discussed below, the upstream gene appears to also encode a redox protein and is tentatively referred to as rubredoxin oxidoreductase, the product of the *rbo* gene.

The nucleotide sequence of the *rub* gene and its upstream region (nt 1301 to 2140) is shown in Fig. 3. Each nucleotide was determined four times on average, at least once on each strand. The gene encoding rubredoxin (nt 1896 to 2057) and the putative *rbo* gene predicted by the codon probability calculations in Fig. 2a (nt 1499 to 1879) have been translated into amino acid sequences. Each gene is preceded by a plausible ribosome-binding site. A search for *E. coli* promoters (Fig. 2d and e) indicates the presence of a promoter consensus sequence (−35, −10) upstream from these two genes, and a potential promoter has been indicated in Fig. 3. A hairpin-loop-forming structure of nine G · C base pairs, which may serve as a transcription terminator, is present immediately downstream from the *rub* gene. Thus, an analysis of the nucleic acid sequence indicates that the two genes may form an operon. Direct experimental evidence for the presence of both genes on a single transcript is presented below.

Northern blotting and S1 nuclease mapping. Northern blots of RNA isolated from both *D. vulgaris* and *E. coli* TG2(pJK29) indicate that a single transcript of approximately 680 nucleotides hybridizes with both the pRbo1 and the pRub1 probes (Fig. 4). The 1.1-kb insert of pJK29 is in the correct orientation for transcription of the *rbo* and *rub* genes from the *E. coli lac* promoter present on the pUC vector. However, a transcript originating from the *lac* promoter and containing both the *rbo* and *rub* genes would be approximately 1,100 nt long, and this size is not consistent with the data in Fig. 4C. The observation of similarly sized transcripts in *D. vulgaris* and *E. coli* indicates that the *D. vulgaris* promoter controlling the transcription of these two genes functions in *E. coli* and may thus resemble the *E. coli* consensus. The observed size of the transcript is sufficiently large to accommodate both the *rbo* and the *rub* genes. Assuming that the transcript terminates at position 2110, on the 3′ side of the hairpin indicated in Fig. 3, the transcriptional start site is placed in the vicinity of position 1430, which is 70 nt upstream from the translational start of the *rbo* gene. The transcriptional start site was next defined more precisely by S1 nuclease mapping. Use of the 913-bp probe gives rise to a major protected fragment of 600 ± 50 nt (average of three experiments), while minor protected fragments of variable, smaller sizes are also observed (Fig. 5). The 600-nt fragment ends at nt 2041 (Fig. 3), indicating a transcriptional start site at nt 1441 ± 50. The presence of a promoter sequence with reasonable homology to the *E. coli* consensus at nt 1355 to 1380 (Fig. 3) suggests a potential start

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                                                     -35             -10
GCATACTGTGCCTGAAAAGTGAATCACCCCGGACACATCTTTCCGCTCATGGGCTGGACAAGCCCAGATTAGGATTTACTCGTAAAAAATCACGCCGGTCATTGAACAGACCGCCGACAT
     1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420

                                                                                rbs       M  P  N  Q  Y  E  I  Y  K  C  I  H  C  G
GCTGTACACAGGTATAAACAGGTATTTCCGGCGGCCATTCCCGCCTTTCAGACCACCGACAACGAACATGGAGGCCCCATGCCCAACCAGTACGAAATCTACAAATGCATCCACTGTGGC
     1430      1440      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540

 N  I  V  E  V  L  H  A  G  G  G  D  L  V  C  C  G  E  P  M  K  L  M  K  E  G  T  S  D  G  A  K  E  K  H  V  P  V  I  E
AACATCGTCGAAGTCCTGCATGCTGGCGGCGGCGACCTCGTGTGCTGCGGCGAACCCATGAAGCTCATGAAGGAAGGCACTTCTGACGGGGCCAAGGAAAAGCACGTGCCGGTCATCGAG
     1550      1560      1570      1580      1590      1600      1610      1620      1630      1640      1650      1660

 K  T  A  N  G  Y  K  V  T  V  G  S  V  A  H  P  M  E  E  K  H  W  I  E  W  I  E  L  V  A  D  G  V  S  Y  K  K  F  L  K
AAGACCGCCAACGGCTACAAGGTCACCGTCGGTTCCGTGGCCCACCCCATGGAAGAGAAGCACTGGATCGAATGGATTGAGCTTGTCGCAGACGGTGTGAGCTACAAGAAGTTCCTGAAG
     1670      1680      1690      1700      1710      1720      1730      1740      1750      1760      1770      1780

 P  G  D  A  P  E  A  E  F  C  I  K  A  D  K  V  V  A  R  E  Y  C  N  L  H  G  H  W  K  A  E  A  *      rbs        M  K
CCCGGCGATGCGCCCGAAGCCGAGTTCTGCATCAAGGCCGACAAGGTCGTCGCCCGCGAATACTGCAACCTGCACGGCCACTGGAAGGCCGAAGCCTAACCCCGAGGAATCCACGATGAA
     1790      1800      1810      1820      1830      1840      1850      1860      1870      1880 AvaI 1890      1900

 K  Y  V  C  T  V  C  G  Y  E  Y  D  P  A  E  G  D  P  D  N  G  V  K  P  G  T  S  F  D  D  L  P  A  D  W  V  C  P  V  C
AAAGTACGTATGCACCGTCTGCGGTTACGAATACGACCCTGCTGAAGGCGACCCCGACAACGGCGTGAAGCCCGGCACCTCGTTCGACGACCTGCCGGCCGACTGGGTATGCCCCGTGTG
     1910      1920      1930      1940      1950      1960      1970      1980      1990      2000      2010      2020

 G  A  P  K  S  E  F  E  A  A  *  *                   ------->                  <-------
CGGCGCCCCCAAGAGCGAATTCGAAGCCGCCTAGTAAGCGCTTTCCATGACGCCGCCGCCGGTTGCCCGTACGGGTTGCCCGCGGCGGCGTTTCTTCGTATAGGGGGGTCATCAGCCCCT
     2030      2040      2050      2060      2070      2080      2090      2100      2110      2120      2130      2140
              EcoRI
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FIG. 3. Nucleotide sequence of the *rbo-rub* operon. The numbering of the sequence is the same as in Fig. 1 and 2. The coding regions of the *rbo* (nt 1499 to 1879) and *rub* (nt 1896 to 2057) genes have been translated with the single-letter amino acid code. Translation stop codons are designated by asterisks. The ribosome-binding sites (rbs) and a plausible promoter (−35, −10) are indicated. A G+C-rich stem-and-loop structure (→←) which could serve as a transcription termination signal is shown.

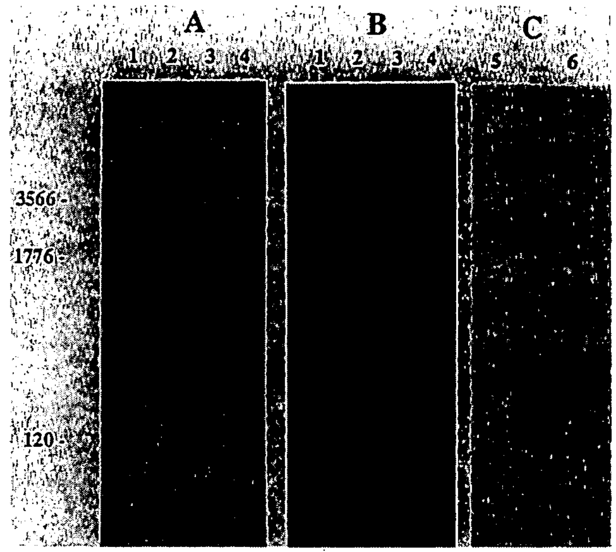

FIG. 4. Northern blotting of total RNA isolated from *D. vulgaris* Hildenborough and *E. coli* TG2(pJK29). (A) Increasing amounts of *D. vulgaris* RNA (25, 50, 75, and 100 μg) were loaded in lanes 1 to 4, respectively. The blot was hybridized to the pRbo1 probe and autoradiographed for 5 days at −70°C. The size markers, 25S rRNA (3,566 bases), 16S rRNA (1,776 bases), and 5S rRNA (120 bases), were visualized by fluorography of the blot following transfer of the ethidium bromide-stained gel. (B) The same blot as in panel A was incubated for 2 min in boiling distilled water to dissociate the pRbo1 probe. Following autoradiography to confirm the dissociation of this probe, the blot was rehybridized to the pRub1 probe. Autoradiography was carried out for 7 days at −70°C. (C) *D. vulgaris* RNA (25 μg) and RNA isolated from *E. coli* TG2 transformed with plasmid pJK29 (100 μg) were loaded in lanes 5 and 6, respectively. Following electrophoresis, the blot was incubated with the pRbo1 probe. Autoradiography was performed for 14 days at −70°C. Use of the pRub1 probe gave identical results (not shown).

site in the vicinity of nt 1390, which is within the range predicted by the S1 nuclease mapping results. The smaller (<600-nt) fragments do not map to the same position and are presumed to be artifacts arising from preferential S1 nuclease cleavage (12).

Southern blotting. Southern blots of chromosomal DNA digested with *Eco*RI from 17 different species and strains of sulfate-reducing bacteria, including *D. vulgaris* Hildenborough, were hybridized with the inserts from plasmids pRub1 and pRbo1 as probes. The results obtained are shown in Fig. 6A and B, respectively, as well as in Table 2. These results are discussed in more detail below.

DISCUSSION

Homology of the *rbo* gene product with desulforedoxin. Desulforedoxin is a small redox protein, which has been isolated from *D. gigas* but has so far not been found in other sulfate-reducing bacteria. Its polypeptide chain is only 36 amino acids long and has been determined by protein sequencing (9). As discussed by Le Gall et al. (18), the protein is isolated as a dimer (molecular mass, 7.9 kDa; 3.9 kDa per subunit) with two bound Fe atoms as redox centres and has rubredoxinlike spectroscopic properties. The product of the *rbo* gene (378 nt) is much larger than desulforedoxin (126 amino acids; molecular mass, 14 kDa). A comparison of the two amino acid sequences shows that the homology resides

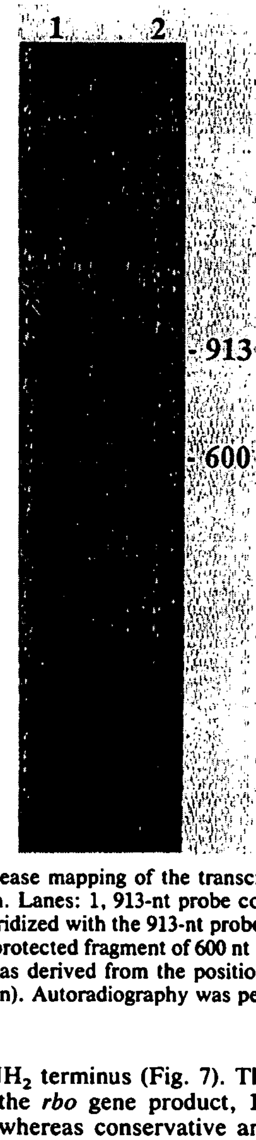

FIG. 5. S1 nuclease mapping of the transcriptional start site of the *rbo-rub* operon. Lanes: 1, 913-nt probe control; 2, *D. vulgaris* RNA (100 μg) hybridized with the 913-nt probe and treated with S1 nuclease; a major protected fragment of 600 nt is indicated. The size of this fragment was derived from the positions of pUC8 × *Hin*fI markers (not shown). Autoradiography was performed for 4 weeks at −70°C.

entirely at the NH_2 terminus (Fig. 7). Thus, of the first 36 amino acids of the *rbo* gene product, 19 are identical to desulforedoxin, whereas conservative amino acid changes are frequently found in the nonidentical positions. The four cysteine residues (C-9, C-12, C-28, and C-29), which have been proposed to coordinate to the iron (18), are among the conserved amino acid residues. Another interesting region of homology comprises the three glycine residues (G-22, G-23, G-24), which must represent an important flexible (e.g., loop) region of the molecule. The high degree of homology indicates that at its NH_2 terminus, the *rbo* gene product must have a 4-kDa desulforedoxin domain, which has been fused to a larger polypeptide of 10 kDa. This situation is very reminiscent to that in [Fe] hydrogenase of *D. vulgaris* Hildenborough, which is known to have three iron-sulfur clusters as prosthetic groups coordinated by cysteine residues of the large (46-kDa) subunit. The NH_2 terminus of this large subunit is homologous to bacterial 8Fe-8S ferredoxin (38), indicating that two electron-transferring 4Fe-4S clusters are likely to be present in this part of the molecule in a structure resembling that of ferredoxin (1), while a third unique hydrogen-binding cluster could be coordinated by some of the 10 cysteine residues found in the 40-kDa COOH-terminal part of the molecule. It was proposed, therefore, that the hydrogenase gene originated from a

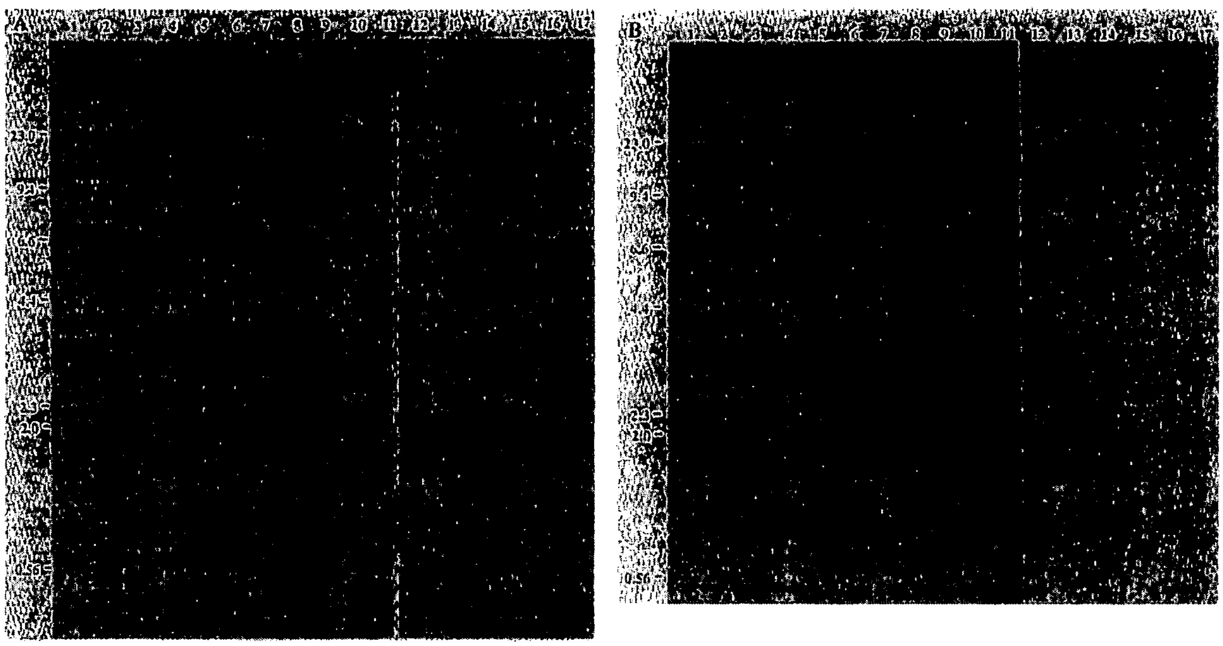

FIG. 6. Southern blotting of *Eco*RI-digested chromosomal DNA (5 to 10 μg) from 17 species and strains of sulfate-reducing bacteria. Lanes: 1, *D. vulgaris* Hildenborough; 2, *D. vulgaris* Wandle; 3, *D. vulgaris* Brockhurst Hill; 4, *D. vulgaris* Groningen; 5, *D. vulgaris* Miyazaki; 6, *D. vulgaris* subsp. *oxamicus* Monticello; 7, *D. desulfuricans* Norway; 8, *D. desulfuricans* Teddington R; 9, *D. desulfuricans* El Agheila Z; 10, *D. desulfuricans* Berre Sol; 11, *D. desulfuricans* Canet 41; 12, *D. gigas*; 13, *D. salexigens* British Guiana; 14, *D. salexigens* California; 15, *D. salexigens*; 16, *D. africanus* Walvis Bay; 17, *D. africanus* Bhengazi. (A) The blots were hybridized with the pRubl probe (containing part of the rubredoxin gene from *D. vulgaris* Hildenborough) and subjected to autoradiography for 8 days at −70°C. The probe was then dissociated from the blots by washing in 0.5 M NaOH–1.5 M NaCl and neutralized by washing with 0.5 M Tris hydrochloride (pH 7.4)–1.5 M NaCl. Complete dissociation of the pRubl probe was confirmed by autoradiography. (B) The blots were then hybridized with the pRbol probe (containing the gene of a putative rubredoxin oxidoreductase from *D. vulgaris* Hildenborough) and subjected to autoradiography for 2.5 days at −70°C. The positions of molecular size markers (bacteriophage λ DNA digested with *Hin*dIII) are indicated in kilobases.

TABLE 2. Hybridization of *Eco*RI-digested chromosomal DNA from 17 different sulfate-reducing bacteria with an *rub*-specific and an *rbo*-specific probe from *D. vulgaris* Hildenborough

Species	Strain	NCIMB no.	Size of hybridizing fragment (kb) for:	
			rub	*rbo*
D. vulgaris subsp. *vulgaris*	Hildenborough	8303	2.0	2.0
	Wandle	8306	2.0	2.0
	Brockhurst Hill	8306	2.0	2.0
	Groningen	11779	15.4	15.4
	Miyazaki		0.5	3.9
D. vulgaris subsp. *oxamicus*	Monticello 2	9442	0.5	1.8
D. desulfuricans subsp. *desulfuricans*	Norway 4	8310		
	Teddington R[a]	8312		7.8
	El Agheila Z	8318		
	Berre Sol	8388	0.5	2.8
	Canet 41	8393	0.5	
D. gigas		9332	1.2	
D. salexigens	British Guiana	8403		
	California 43:63	8364		
		8365		
D. africanus	Walvis Bay	8397	7.1	
	Bhengazi	8401	0.4	3.0

[a] Reclassified as *D. vulgaris* (28).

fusion of genes encoding an electron-transferring ferredoxin and a larger hydrogen-binding polypeptide (38). In the present case, the desulforedoxin domain coincides precisely with the 36 NH_2-terminal amino acids.

Another example of a redox protein that could have arisen by gene fusion is rubrerythrin, which was recently isolated (19) and shown to contain both rubredoxinlike and hemerythrinlike redox centres. The putative *rbo* gene product is distinct from rubrerythrin in its molecular mass (14 versus 22 kDa) and amino acid composition. Also, preliminary protein sequence data on rubrerythrin (D. M. Kurtz, Jr., and J. Le Gall, personal communication) indicate that this protein does not resemble the *rbo* gene product.

Two possible modes of coordination of the two iron atoms by the eight cysteines of a desulforedoxin dimer have been considered by Le Gall et al. (18): the four cysteines of a single subunit could coordinate to the same iron, in which case dimer formation is achieved by noncovalent interactions of the two subunits, or cysteines from both subunits (e.g., C-9, C-12, and C-28 from one and C-29 from the other) could contribute to the coordination of the two irons, causing a partially covalent connection of the two subunits via the two Fe redox centres. In view of its homology with desulforedoxin, the functional form of the *rbo* gene product is also proposed to be a dimer. Only two additional cysteine residues (C-103 and C-115) are present in the remainder of the sequence of the *rbo* gene product (Fig. 7), which is insufficient for the coordination of a third Fe-containing redox centre (e.g., the coordination of a single Fe, a single

```
                5                   10                  15
pro-asn-gln-tyr-glu-ile-tyr-lys-cys-ile-his-cys-gly-asn-ile-
ala-asn-glu-gly-asp-val-tyr-lys-cys-glu-leu-cys-gly-gln-val-

               20                  25                  30
val-glu-val-leu-his-ala-gly-gly-gly-asp-leu-val-cys-cys-gly-
val-lys-val-leu-glu-glu-gly-gly-gly-thr-leu-val-cys-cys-gly-

               35                  40                  45
glu-pro-met-lys-leu-met-lys-glu-gly-thr-ser-asp-gly-ala-lys-
glu-asp-met-val-lys-gln

               50                  55                  60
glu-lys-his-val-pro-val-ile-glu-lys-thr-ala-asn-gly-tyr-lys-

               65                  70                  75
val-thr-val-gly-ser-val-ala-his-pro-met-glu-glu-lys-his-trp

               80                  85                  90
ile-glu-trp-ile-glu-leu-val-ala-asp-gly-val-ser-tyr-lys-lys-

               95                 100                 105
phe-leu-lys-pro-gly-asp-ala-pro-glu-ala-glu-phe-cys-ile-lys-

              110                 115                 120
ala-asp-lys-val-val-ala-arg-glu-tyr-cys-asn-leu-his-gly-his-

              125
trp-lys-ala-glu-ala
```

FIG. 7. Comparison of the amino acid sequence of the *rbo* gene product (Fig. 3) with that of desulforedoxin (9). Regions of sequence identity are highlighted by boxes.

2Fe-2S cluster, or a single 4Fe-4S cluster requires four cysteines). However, a third redox centre could be present in the 14-kDa *rbo* gene product, if one assumes that the two subunits can donate both cysteine ligands to the same coordination site. The *rbo* gene product appears quite different from the NADH-rubredoxin oxidoreductase purified from *C. acetobutylicum* which was discussed in the Introduction. The subunit molecular mass is different (41 kDa for the *Clostridium* enzyme), and the search of the Bionet data base did not reveal homologies of the *rbo* gene product with flavin-binding proteins.

In analogy with the [Fe] hydrogenase gene, one could suggest that the *rbo* gene has arisen by fusion of the gene for desulforedoxin with that encoding a 10-kDa redox protein containing a single redox centre. The observation that this gene forms a single operon with the gene encoding rubredoxin could mean that the two proteins are redox partners, but definitive proof for this proposal must await the purification and characterization of this novel redox protein.

Distribution of the *rbo* gene in sulfate-reducing bacteria. The *rub* and *rbo* genes are present on a similarly sized *Eco*RI fragment (Fig. 6) and may thus form an operon as in Fig. 3 in *D. vulgaris* Wandle, Brockhurst Hill, and Groningen. This is no surprise for the first two strains, which are practically identical to the Hildenborough strain (39). However, the Groningen strain is quite distinct from Hildenborough and was shown to lack a gene for [Fe] hydrogenase and to have only a low degree of homology in its gene for cytochrome c_3 (39). Four species of sulfate-reducing bacteria have homologous *rub* and *rbo* genes on different restriction fragments (Table 2; Fig. 6). This could indicate a separation of the two genes in these species or the presence of an additional *Eco*RI site in their *rbo-rub* operon. Finally, a homologous *rub* gene in the absence of a detectable, homologous *rbo* gene is found in three, whereas the converse is found in a single one of the species of sulfate-reducing bacteria examined. *D. gigas*, which has a rubredoxin with 71% sequence identity (37 identical residues in a total of 52) with the protein from *D. vulgaris* Hildenborough (36), the pRub1 probe appears sufficiently homologous for the detection of the *rub* gene on a 1.2-kb *Eco*RI fragment (Fig. 6A, lane 12; Table 2). However, the 53% identity of *D. gigas* desulforedoxin with the N terminus of the *D. vulgaris rbo* gene product (Fig. 7; 19 identical residues in a total of 36) appears insufficient for the detection of the desulforedoxin gene in *D. gigas* (Fig. 6B, lane 12). The data in Fig. 6 should therefore not be interpreted in terms of the presence or absence of a gene encoding a desulforedoxinlike polypeptide in species that fail to hybridize with the pRbo1 probe. They indicate that the *rbo* gene is present in nine other species of sulfate-reducing bacteria and is spatially linked to the *rub* gene in at least four species. The *rbo* gene product could thus function as a redox protein in some but not all species of sulfate-reducing bacteria.

ACKNOWLEDGMENTS

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THE CHELATE STRUCTURE OF MICROCOCCUS AEROGENES RUBREDOXIN*

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Rubredoxin represents the simplest type of nonheme iron protein, as it is a small molecule (mol wt 6000) containing only 1 gm atom of iron and no "labile sulfur."[1] In addition, it lacks the amino acids histidine and arginine[1,2] which are potential ligands of iron. A knowledge of the amino acid sequence of *Micrococcus aerogenes* rubredoxin[2] has aided in the study of the chelate structure. Previous investigations have demonstrated that the iron in rubredoxin exists in the form of an octahedral complex in which four of the ligands are cysteine residues.[3] In the present communication, results are presented concerning the nature of the fifth and sixth ligand of the ferric iron.

This paper describes various chemical modifications of native rubredoxin and its apoprotein. The ability of modified apoprotein to reconstitute with ferric iron was followed by absorption and electron paramagnetic resonance (EPR) spectra.

Materials and Methods.—Preparation of rubredoxin and derivatives: The procedure for isolation of purified rubredoxin has been previously reported.[3] The preparations used in the present study possessed an $E_{280m\mu}/E_{490m\mu}$ ratio of 2.4. The apoprotein was prepared by precipitation with trichloroacetic acid as previously described.[3] Protein concentrations were determined by amino acid analyses of aliquots of stock solutions after hydrolyzing the samples for 24 hr in 6 N HCl.

Reconstitution of rubredoxin: The lyophilized apoprotein (0.05–0.1 μmole) was dissolved in 0.9 ml of 1 M NaCl–0.04 M veronal–HCl buffer, pH 7.5. To this solution 0.1 ml of dithiothreitol (aqueous solution, 0.5 M) was added and the mixture incubated at 40° for 5 min. Afterward, 0.01 ml of a neutral $FeCl_3$ solution (0.5 mg Fe/ml) was added, and the mixture maintained at 40° for a further 10 min.

Reaction with 2-hydroxy-5-nitrobenzylbromide:[4] Dried aporubredoxin (0.05–0.1 μmole) was dissolved in 0.8 ml of acetate buffer (0.17 M, pH 4.6) and reacted with a 70-fold molar excess of 2-hydroxy-5-nitrobenzylbromide in acetone. The reaction was allowed to proceed in the dark for 2 hr at 25°. Excess reagent was removed by passing the reaction mixture through a column of Sephadex G-25.

Dinitrophenylation: Dinitrophenylation[5] of the native protein (0.022 μmole) was carried out in 4% $NaHCO_3$, in $NaHCO_3$–Na_2CO_3 buffer, pH 10, or in a 4% $NaHCO_3$–50% ethanol mixture using a 2000-fold excess of fluorodinitrobenzene (FDNB) in a total volume of 0.2 ml. Reactions were carried out in the dark for 2 hr at 25° or 60°. The reagent and side products of the reaction were removed by gel filtration on Sephadex G-25.

Oxidation of tryptophan with N-bromosuccinimide (NBS):[6] The NBS used was recrystallized from water. For the reaction, 0.1 μmole of rubredoxin or aporubredoxin was dissolved in 0.9 ml of 8 urea–0.33 M acetate buffer, pH 4.0. Increasing amounts of fresh solution (0.015 M) of NBS in water were added and the change in absorbance at 280 and 490 mμ were recorded.

Acetylation with N-acetyl imidazole:[7] About 0.1 μmole of native rubredoxin or aporubredoxin was reacted with a 60-fold excess of N-acetyl imidazole (K and K Lab.) in 0.9 ml of 0.05 M veronal-HCl, pH 7.5, containing 1 M NaCl. The reaction was carried out at 25° for varying periods of time. The acetylated protein was purified on Sephadex

G-25. In the experiments with C-14-labeled reagent (Cal Biochem, sp. act. 22.28 mc/mM), identical conditions were used and after purification on a column of Sephadex G-25, radioactivity was measured in a liquid scintillation counter. The acetyl groups were removed by the addition of 0.1 ml of a 10% solution of hydroxylamine-HCl adjusted to pH 7.5 with NaOH and incubating for 15 min at 25°.

Reaction with O-methyl isourea:[8] In a typical experiment, 0.066 μmole of aporubredoxin, dissolved in 0.1 ml of 0.2 *M* Na_2CO_3, was reacted with 0.05 ml of 1 *M* *O*-methyl isourea (K and K Lab.). In some experiments 95% ethanol was added to the reaction mixture to make the solution 50% with respect to ethanol. The reactions were carried out for 4–7 days at 4°. The excess reagent was removed by passing the reaction mixture through Sephadex G-25. The homoarginine content was determined in acid hydrolysates (24 hr) on the short column of the automatic amino acid analyzer.

Results.—Experiments with native rubredoxin: The reaction of chemical reagents with *M. aerogenes* rubredoxin was investigated to determine the possible ligands chelated to iron. The loss in color of the red protein was used to determine whether or not the chelate structure in the native protein was altered by the chemical reagents. Of course, the fact that the reagent may be altering groups involved in maintaining the secondary and tertiary structure and thus indirectly causing an alteration of the chelate structure must be kept in mind.

Possible chelation of the iron with the -NH_2 groups: The reaction of rubredoxin with fluorodinitrobenzene under various conditions is summarized in Table 1.

TABLE 1. *Reactivity of native rubredoxin with FDNB.*

Sample	Reaction conditions*	Reacted Residues of— Lysine	Tyrosine
A	4% $NaHCO_3$	1.10	0.50
B	Carbonate buffer, pH 10	1.62	1.00
C	1% $NaHCO_3$, 60°	1.73	2.00
D	1% $NaHCO_3$, 50% ethanol	1.60	2.60

* About 0.022 μmole of rubredoxin were used in each case. After the reaction was concluded, the reaction mixture was passed through a column of Sephadex G-25. The DNP-protein was then hydrolysed with 6 *N* HCl for 18 hr and the amino acid content was determined with an automatic amino acid analyzer. From the known content of the various amino acids in native rubredoxin, the loss of the residues was assumed to be due to reaction with FDNB.

Since the yellow color of the DNP-protein obscured the visible spectrum of the protein, the EPR spectra of the reaction products were investigated, as previously described,[3] to determine whether or not the chelate structure was altered in the reaction. In experiment B, when both lysine residues and one of the tyrosines had reacted, the EPR spectrum was similar to that of the native protein. However, in experiment D, where the dinitrophenylation was carried out in the presence of 50 per cent ethanol, the EPR spectrum characteristic of the native protein was abolished. The two residues of lysine were again found to have reacted but in this case all three tyrosine residues were converted to the *O*-DNP-derivative. These experiments ruled out lysine as one of the potential ligands and suggested the possibility that one or both of the tyrosine residues, reacting with FDNB only in the presence of 50 per cent ethanol, might be linked to the iron.

Possibility of methionine as a ligand: Treatment of the rubredoxin with cyanogen bromide (CNBr)[9] at pH 3 released 20–30 per cent of the NH_2-terminal methionine as homoserine. In a separate experiment, after the digestion of the native

rubredoxin with leucine aminopeptidase, 20 per cent of the methionine and 17 per cent of the glutamine were found to be released. Neither the absorption spectrum nor the extinction coefficient of the rubredoxin solutions was altered in any of the above-mentioned experiments. Thus, these experiments ruled out a bond between the iron and the sulfur atom or the α-NH_2 group of the methionine.

Possible chelation of the iron with the tryptophan residue: N-bromosuccinimide readily bleached the spectrum of rubredoxin. The velocity of the reaction depended on the amount of reagent used. In 0.33 *M* acetate buffer, pH 4, containing 8 *M* urea, the absorbance at 490 mμ dropped 75 per cent after three hours of reaction at a NBS-to-rubredoxin ratio of 1:1. At a ratio of 2.2:1, a drop of 96 per cent of the 490 mμ peak was observed after 2½ hours, as shown in Figure 1.

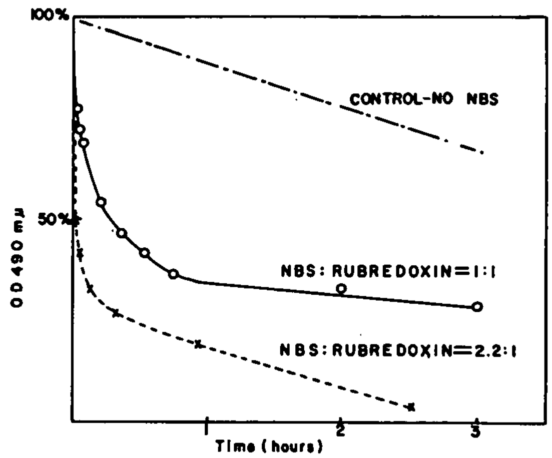

FIG. 1.—Decolorization of rubredoxin with N-bromosuccinimide (NBS). The degree of deterioration was measured by the drop of the OD_{490} as a function of reaction time at room temperature for different ratios of reagent to rubredoxin.

According to Funatsu *et al.*,[6] under these conditions, the only residue which should react significantly is tryptophan. The experiments suggested a need for further investigations of the possible role of tryptophan as a potential candidate in iron binding.

Experiments with aporubredoxin: Aporubredoxin was readily prepared by incubation with trichloroacetic acid. It was possible to reconstitute it 85–100 per cent by the addition of dithiothreitol and $FeCl_3$ (see *Materials and Methods*). The fully reconstituted protein had an absorption spectrum and EPR signal identical to that of the native protein. As a consequence of this finding, the reconstitution of modified apoprotein with iron could be investigated as well.

Acetylation of the tyrosine residues of aporubredoxin: The hydroxyl groups of the three tyrosine residues in aporubredoxin were acetylated by reaction with *N*-acetyl imidazole. The extent of the reaction was followed by using *N*-acetyl imidazole (acetyl-C-14) or by following the decrease in absorbancy at 280 mμ. The acetylated derivatives could no longer be reconstituted. Removal of the acetyl groups with hydroxylamine restored the ability of the apoenzyme to recombine with $FeCl_3$, as shown in Figure 2.

Possible involvement of tryptophan: Aporubredoxin was reacted with NBS in 8 *M* urea, pH 4.0. From the drop in absorbance at 280 mμ, about 90 per cent of

the tryptophan residue was converted to the oxindole-derivative. The reaction product was purified on a column of Sephadex G-25 and the reconstitution experiment was attempted but only 10 per cent of the derivative was reconstitutable.

Another sample of aporubredoxin was reacted with 2-hydroxy-5-nitrobenzyl-bromide and the one tryptophan residue was substituted. This derivative could

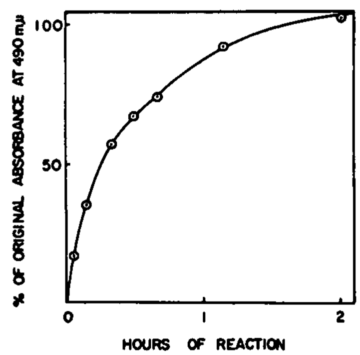

FIG. 2.—Deacetylation of acetyl rubredoxin with hydroxylamine. The reaction mixture contained 0.05 μmole aporubredoxin, dissolved in 1.0 ml veronal-HCl, pH 7.5, which contained 1 M NaCl. After 30 min of reaction with a 60-fold molar excess of N-acetyl imidazole, Cleland's reagent and $FeCl_3$ were added, as described for the reconstitution experiment in the experimental part. No color developed. Then 0.1 ml of 10% hydroxylamine (pH adjusted to 7.5) was added to the reaction mixture and the development of color followed as a function of time.

not be reconstituted. These experiments implicated tryptophan as a potential ligand of the iron.

Conversion of lysine to homoarginine: The two lysine residues of rubredoxin were converted to homoarginine by reacting aporubredoxin with O-methyl isourea. About 75–80 per cent of the lysine residues reacted. The derivatives could still be reconstituted to rubredoxin to the same extent as the untreated apoprotein.

Discussion.—Two approaches were used to determine the ligands 5 and 6 chelated to iron, in addition to the four cysteine residues, in the octahedral chelate structure present in rubredoxin. The first approach involved modifications of native rubredoxin with various chemical reagents and their influence on the spectral properties. A loss of the characteristic absorption spectrum, especially the maxima at 380 and 490 mμ, and of the EPR signal were used as criteria for changes of the iron complex. These studies do not necessarily prove that a specific functional group is linked to the iron since the substitution may have altered the secondary and tertiary structure of rubredoxin. Nonetheless, experiments with suitable tyrosine and tryptophan reagents indicated that a tyrosine phenolic group and the indole nitrogen of the sole tryptophan residue are linked to the iron.

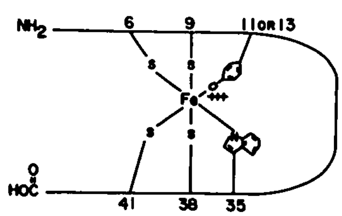

FIG. 3.—Schematic drawings of the possible ligands of rubredoxin. Numbers indicate position of amino acid residues in the peptide chain.

A more direct approach was made possible by the preparation of aporubredoxin which could be reactivated by reducing agents plus ferric iron. An important point is the fact that many of the functional groups are shielded in the native rubredoxin. However, the apoprotein reacts readily with most chemical reagents, suggesting a more open confirmation. The ability of the apoprotein

to recombine with ferric iron under suitable conditions is abolished by reagents which are known to modify tyrosine hydroxyl groups and the indole ring of tryptophan. From similar experiments with reagents specifically modifying the ϵ-NH_2 group of the lysine residues, this amino acid could definitely be excluded as an iron ligand. Consequently, we propose the chelate structure in Figure 3 with a phenolic hydroxyl group and the nitrogen of the indole ring as fifth and sixth ligand of the iron chelate of *M. aerogenes* rubredoxin.

This view is supported by the homology in the amino acid sequence of two different rubredoxins. In both *M. aerogenes* and *Peptostreptococcus elsdenii* rubredoxin[10] the sole tryptophan residue occupies position 35, and tyrosine residues are found in positions 11 and 13. On the other hand, the tyrosine in position 50 of *M. aerogenes* rubredoxin is replaced by a lysine residue in the *P. elsdenii* protein which makes it unlikely that this tyrosine is bound to the iron.

Summary.—The two lysine residues and the NH_2-terminal methionine are definitely not linked to the iron in the chelate structure of *M. aerogenes* rubredoxin. Evidence is presented indicating that ligands 5 and 6 are the phenolic hydroxyl group of a tyrosine residue (residues 11 or 13) and the indole nitrogen of the tryptophan residue in position 35. The evidence is based on the reaction of group-specific chemical reagents with native rubredoxin and its apoprotein.

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Characterization of Ferredoxin, Flavodoxin, and Rubredoxin from *Clostridium formicoaceticum* Grown in Media with High and Low Iron Contents

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Ferredoxin, flavodoxin, and rubredoxin were purified to homogeneity from *Clostridium formicoaceticum* and characterized. Variation of the iron concentration of the growth medium caused substantial changes in the concentrations of ferredoxin and flavodoxin but not of rubredoxin. The ferredoxin has a molecular weight of 6,000 and is a four iron-four sulfur protein with eight cysteine residues. The spectrum is similar to that of other ferredoxins. The molar extinction coefficients are 22.6×10^3 and 17.6×10^3 at 280 and 390 nm, respectively. From 100 g wet weight of cells grown with 3.6 μM iron and with 40 μM iron, 5 and 20 mg of ferredoxin were isolated, respectively. The molecular weight of rubredoxin is 5,800 and it contains one iron and four cysteines. The UV-visible absorption spectrum is dissimilar to those of other rubredoxins in that the 373 nm absorption peak is quite symmetric, lacking the characteristic 350-nm shoulder found in other rubredoxins. The flavodoxin is a 14,500-molecular-weight protein which contains 1 mol of flavin mononucleotide per mol of protein. It forms a stable, blue semiquinone upon light irradiation in the presence of EDTA or during enzymatic reduction. When cells were grown in low-iron medium, flavodoxin constituted at least 2% of the soluble cell protein; however, it was not detected in extracts of cells grown in high-iron medium. The rubredoxin and ferredoxin expressed during growth in low-iron and high-iron media are identical as judged by iron, inorganic sulfide, and amino acid analysis, as well as light absorption spectroscopy.

Ferredoxin, flavodoxin, and rubredoxin are low-molecular-weight electron-carrier proteins which are important in anaerobic metabolism. In the metabolism of the acetogenic bacteria, ferredoxin is an electron acceptor for numerous low-potential oxidation-reduction reactions, including pyruvate:ferredoxin oxidoreductase (9, 38, 44), hydrogenase (8; Pezacka and Wood, Arch. Microbiol., in press), NADH:ferredoxin oxidoreductase (38, 44), and CO dehydrogenase (6, 17, 34). Flavodoxin has been shown to substitute for ferredoxin in many redox reactions, but at a slower rate of electron transfer (20, 21). Rubredoxin has been found to substitute for ferredoxin in a few oxidation-reduction reactions, but usually at a very low rate, since the redox potential of rubredoxin is approximately 400 mV higher than that of ferredoxin. Recently, rubredoxin was found to be a superior electron acceptor in the CO dehydrogenase reaction in acetogenic bacteria (33, 34). The properties of rubredoxin have been reviewed (10).

We studied these three electron carriers in *Clostridium formicoaceticum* since flavodoxin has not been isolated from acetogenic bacteria and ferredoxin (12, 44) and rubredoxin (43) have been purified from only one acetogen, *Clostridium thermoaceticum*. Characterization of the electron carriers involved in the acetate biosynthetic pathway is important, since the acetogens carry out a reduction of CO_2 to acetate in a sequence of reactions of four two-electron transfers from some electron donor (CO, H_2, pyruvate, glucose, etc.) to a pathway involving formate dehydrogenase (42) and tetrahydrofolate enzymes (22).

MATERIALS AND METHODS

Thioglycolic acid, DEAE-Sephadex A50-120, and Sepharose CL-6B-200 were obtained from Sigma Chemical Co. Ultrapure hydrochloric acid was from Alfa Thiokol/Ventrol. Ultrogel AcA chromatography gels were from LKB Instruments and Sephadex gels were from Pharmacia. DEAE-cellulose was from Whatman Ltd.

Growth of *C. formicoaceticum*. *C. formicoaceticum* ATCC 23439, described by Andreesen et al. (1), was maintained and grown by using the medium of Moore et al. (29) containing 3.6 μM iron as described earlier (2). When high-iron medium was used, the concentration of ferrous ammonium sulfate was 40 μM.

Analytical methods. Protein concentration was determined by the rose bengal dye-binding assay (11), using ovalbumin as standard, or by the extinction coefficients, which were determined as described below. Amino acid analysis was carried out on the homogenous electron carrier proteins by first dialyzing samples for 36 h against double-distilled water and by hydrolyzing in vacuo in ultrapure 6.0 N HCl for 12, 24, 48, and 72 h at 110°C. Tryptophan was determined by acid hydrolysis in 4.0 and 6.0% thioglycolic acid (26), and cysteine was determined as cysteic acid (16). Norleucine was included as internal standard. The partial specific volume was calculated from the amino acid composition (3). The extinction coefficients were calculated by using amino acid analyses to determine the nanomoles of protein relative to the absorbance at a given wavelength. The rose bengal protein assay was standardized for each protein by amino acid analysis.

Sedimentation equilibrium and velocity experiments were performed with a Beckman model E ultracentrifuge equipped with absorption optics. In velocity experiments, the sedimentation coefficient was determined by the moving boundary method (35), using the schlieren peak to detect the position of the boundary as a function of time. Temperature was maintained at 20°C during the velocity and equilibrium experiments. All samples were run in 50 mM potassium phosphate–0.3 M potassium chloride buffer. The sedimentation coefficient was corrected to the condition of water at

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TABLE 1. Properties of ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum*

Protein	Mol wt determined by: Gel filtration	Sedimentation equilibrium	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis	Amino acid analysis	Partial specific volume[a]	Stokes radius[b]	Iron per mol of protein[c]
Ferredoxin	5,200 ± 500	6,700 ± 200		6,000 ± 100	0.663	9.5 ± 0.4	4.6 ± 0.4
Rubredoxin	5,600 ± 500	5,900 ± 100		5,800 ± 50	0.715	10.0 ± 0.4	1.2 ± 0.2
Flavodoxin	14,000 ± 1,000	16,500	14,500 ± 500	14,000 ± 1,000	0.731	17.4 ± 0.7	ND

20°C. The molecular weights were calculated according to the Svedberg equation (35), assuming a one-component system.

The Stokes radii and molecular weights were determined by using carbonic anhydrase, cytochrome *c*, ovalbumin, chymotrypsinogen A, and RNase A as standards. The correlation coefficient of the line obtained in the standardization was 0.992.

Acid-labile sulfide was analyzed (32) by using sodium sulfide as the standard. Iron was determined with bathophenanthroline (7) and by plasma emission spectroscopy (19). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done by the method of Weber et al. (41), and standard alkaline electrophoresis was performed with Tris-glycine (5). Preparative electrophoresis was carried out with an LKB 7900 Uniphor system with pH 8.0 Tris-barbital (14).

Flavodoxin was immobilized to Sepharose CL-6B as described by Mayhew and Strating (28) after cyanogen bromide activation (4).

Purification of flavodoxin, rubredoxin, and ferredoxin from *C. formicoaceticum* cells. Figure 1 gives a summary of the purification procedure. This procedure describes the purification from low-iron-grown cells; however, identical steps were used in the purification of rubredoxin and ferredoxin from high-iron-grown cells.

Crude extract. Frozen cells (205 g) were suspended in 600 ml of 50 mM potassium phosphate buffer, pH 7.2, and extracted as described previously (2). The suspension was centrifuged in a type 35 Beckman rotor at 57,000 × *g* for 90 min. The supernatant (21,000 mg of protein) was used in the purification procedure.

DE23 cellulose. The supernatant of the ultracentrifugation step was applied to a DE23 cellulose column (5.5 by 38 cm) which was washed with 50 mM potassium phosphate, pH 7.2. The top layer of this column containing the electron carriers was removed with a spatula and placed on a fresh bed of DE23 cellulose equilibrated with Tris-hydrochloride (0.2 M, pH 7.6). The column was washed with 0.25 M Tris-

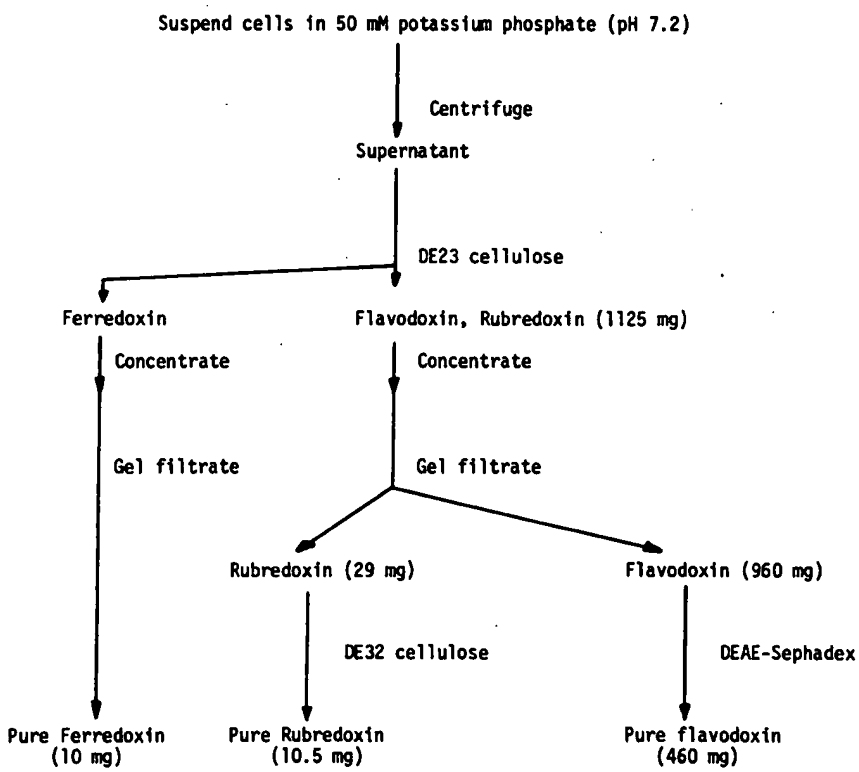

FIG. 1. Purification of rubredoxin, flavodoxin, and ferredoxin from 205 g of *C. formicoaceticum* cells grown in low-iron medium. The final yields of ferredoxin and flavodoxin were dependent upon the concentration of iron in the medium. See the text for details.

TABLE 1. (Continued)

Sulfur per mol of protein	Sedimentation coefficient ($S_{20,w}$)	Molar extinction coefficient (liter mol^{-1} cm^{-1}) at 280 nm	390 nm	490 nm	272 nm	373 nm	444 nm
4.3 ± 0.4	ND[d]	22,600	17,600				
0	ND	18,200		7,300		9,200	
0	2.1	ND			38,600	6,500	7,700

[a] Determined from amino acid analysis.
[b] Determined from gel filtration.
[c] Average of iron determinations by the Doeg-Ziegler method and by plasma emission spectroscopy, using the amino acid analysis for the determination of the protein concentration.
[d] ND, Not determined.

hydrochloride extensively before the flavodoxin and rubredoxin were eluted together with 0.3 M Tris-hydrochloride. The 2-liter eluate was concentrated to 50 ml by using a DE32 cellulose column (2.6 by 20 cm) and then to 15 ml with an Amicon UM-2 ultrafiltration membrane. This concentrate contained 1,125 mg of protein when cells grown in low-iron medium were used. Ferredoxin was eluted from the first DE23 column after the elution of flavodoxin and rubredoxin with 0.5 M Tris-hydrochloride.

Tandem gel filtration. The rubredoxin-flavodoxin concentrate from the DE23 step was applied to a tandem AcA 54-AcA 44 column setup (both 2.6 by 85 cm). When run in low-salt buffers, flavodoxin seemed to aggregate, so 0.2 M KCl was included in the 50 mM Tris buffer. Under these conditions, flavodoxin eluted in a tight band. The flavodoxin solution contained 960 mg of protein, whereas the rubredoxin eluate had 29 mg of protein. Rubredoxin eluted well-separated from flavodoxin.

The ferredoxin from DE32 was applied to the same gel filtration setup and run in the same buffer system. The resulting ferredoxin was pure as judged by the absorption ratio (absorbance at 390 nm [A_{390}]/A_{280}), and by sedimentation velocity and equilibrium experiments.

DE32 cellulose. Rubredoxin from the tandem column was adsorbed to a 5-ml DE32 cellulose column equilibrated with Tris-hydrochloride (0.1 M), and a 200-ml linear gradient from 0.2 to 0.5 M Tris-hydrochloride was run. The red, rubredoxin-containing fraction was concentrated on a small DE32 cellulose column from 75 to 2.5 ml. The yield of rubredoxin was 10.5 mg with an absorption ratio (A_{280}/A_{490}) between 2.35 and 2.50.

The rubredoxin from this step was judged to be pure by sodium dodecyl sulfate and alkaline electrophoresis, sedimentation velocity, and absorbance ratio. In sedimentation equilibrium experiments the ln of the concentration versus r^2 plot was linear, and the molecular weights calculated with both 490- and 280-nm scans were equivalent, which also indicates homogeneity.

DEAE-Sephadex chromatography and preparative electrophoresis. Final purification of flavodoxin could be accomplished by either DEAE-Sephadex chromatography or preparative electrophoresis. Flavodoxin from the tandem column was absorbed to a DEAE-Sephadex column (2.6 by 22 cm) in 50 mm Tris-hydrochloride (pH 7.6) and eluted with a 2-liter gradient of 0 to 0.25 M sodium chloride in 0.1 M Tris-hydrochloride. The yield of flavodoxin was 460 mg with an absorption ratio (A_{272}/A_{373}:A_{444}/A_{373}) of 6.36:1.28. Preparative electrophoresis, described above, yielded a protein with the same characteristics as that obtained in the DEAE-Sephadex step. It was essential to use the pH 8.0 Tris-barbital system in preparative electrophoresis since the Tris-glycine pH 8.9 system caused loss of the flavodoxin cofactor, flavin mononucleotide. Dissociation of flavin mononucleotide from the protein is reversible, however, as shown below. The protein from this step was homogenous as judged by alkaline and sodium dodecyl sulfate electrophoresis and sedimentation equilibrium and velocity experiments.

RESULTS

Effect of iron on the relative amounts of electron carriers. When grown in medium containing only 3.6 μM iron salts, flavodoxin is a major component of the soluble cell protein. Approximately 2% of the soluble cell protein (230 mg per 100 g wet weight of cells) was recovered as homogenous flavodoxin. Under these iron-poor conditions, approximately 5 mg of ferredoxin could be recovered per 100 g wet weight of cells. When the medium was supplemented with 40 μM of iron, the amount of ferredoxin that could be recovered as the homogenous protein increased four- to fivefold. Flavodoxin was undetectable in extracts of high iron-grown cells. Rubredoxin amounted to 3 to 5 mg per 100 g of cells, and the amount was unaffected by addition of supplemental iron to the growth medium.

Properties of electron transfer proteins from *C. formicoaceticum*. The properties of the electron transfer proteins are summarized in Table 1. The amino acid compositions, iron and inorganic sulfur contents, and molecular weights of ferredoxin, rubredoxin, and flavodoxin were the same regardless of whether the cells were grown in high- or low-iron medium. Only one type of ferredoxin or rubredoxin was seen during chromatography in the purification procedures for high- or low-iron-derived proteins, which is in contrast to results with *C. thermoaceticum*, which contains two ferredoxins (12, 44) and two rubredoxins (43).

Ferredoxin. The average molecular weight of ferredoxin, calculated from gel filtration, amino acid analysis, and sedimentation equilibrium, was 6,000. Amino acid analysis (Table 2) gave eight cysteines and a partial specific volume of 0.663 ml/g. By both plasma emission spectroscopy and the method of Doeg and Ziegler (7), approximately four irons were found per mole of protein. Four acid-labile sulfides were found per mole protein. The molar extinction coefficients were 22,600 at 280 nm and 17,600 at 390 nm. It was essential to determine protein concentration by using amino acid analysis including an internal standard. Use of the rose

TABLE 2. Amino acid analysis of ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum*

Amino acid	Ferredoxin	Rubredoxin	Flavodoxin
Asx	8	8	7
Thr	2	2	4
Ser	2	1	3
Glx	5	5	28
Pro	4	6	4
Gly	4	6	21
Ala	8	3	9
Cys	8	4	1
Val	5	6	8
Met	1	1	9
Ile	4	2	3
Leu	0	0	14
Tyr	2	3	2
Phe	0	2	2
His	0	0	0
Lys	1	3	8
Arg	0	0	2
Trp	0	1	1

bengal (11) or Lowry et al. (24) assay gave values for protein concentration that were seriously in error. The purified ferredoxin had an absorbance ratio (A_{390}/A_{280}) of between 0.74 to 0.78 and a spectrum similar to those of other ferredoxins (30).

Rubredoxin. The average molecular weight of rubredoxin was 5,800. As in other rubredoxins, four cysteines were found. The number of irons per mole was approximately 1 to 1.5; however, since the extinction coefficients, absorption ratio (A_{280}/A_{490}), and cysteine content were so similar to those of the one-iron rubredoxin, we feel that nonessential iron must be absorbed to the protein. Plasma emission spectroscopy revealed the presence of 3.0 Ca, 8.6 K, 4.2 Na, and 2.0 P per mol protein, so it is reasonable that nonfunctional iron could also be bound. By absorbing the purified rubredoxin to DEAE-cellulose and then washing with pH 5.5 sodium citrate buffer (0.1 M), lower iron readings (approximately 1.0 mol of Fe per mol of protein) than those found in the nontreated sample were obtained.

The spectrum of rubredoxin was unlike that of other rubredoxins (Fig. 2) in that the 373-nm peak was quite symmetric. Thus, the *C. formicoaceticum* rubredoxin lacks a shoulder at 350 nm that is characteristic of other rubredoxins (10, 23, 43). The same spectrum was obtained from rubredoxins isolated from both high- and low-iron-derived cells.

Rubredoxin from *C. formicoaceticum* contains no histidine or arginine (Table 2), a common characteristic of rubredoxins. Tryptophan also is present in this rubredoxin. The partial specific volume is 0.715 ml/g.

Flavodoxin. The average molecular weight of flavodoxin is 14,500. Extinction coefficients are shown in Table 1. A partial specific volume of 0.730 ml/g was obtained from the amino acid composition (Table 2). The spectrum (Fig. 3) of the stable semiquinone formed by treatment with light in the presence of EDTA (25) or CO plus CO dehydrogenase was very similar to that of the *Megasphaera elsdenii* flavodoxin (27). The cofactor was identified as flavin mononucleotide by coupling the protein to a Sepharose-CL-6B and eluting cofactor with 5% trichloracetic acid plus 0.3 mM EDTA as described by Mayhew et al. (28). Flavin adenine dinucleotide, flavin mononucleotide, and riboflavin were equilibrated with the column, but only flavin mononucleotide would bind to the Sepharose-bound apoprotein.

DISCUSSION

The concentration of iron in the growth medium significantly affects the relative concentrations of ferredoxin and flavodoxin, but not of rubredoxin, in cells of *C. formicoaceticum*. Similar findings have been reported for *M. elsdenii* (27) and *Clostridium pasteurianum* (20). With *C. pasteurianum*, the maximal amount of ferredoxin is formed by bacteria grown in media containing 40 μM of iron salts (20). From 100 g of *C. formicoaceticum* cells grown in 40 μM iron medium we isolated over 20 mg of ferredoxin but found no evidence for a flavodoxin. In contrast, from the same amount of cells grown with 3.6 μM iron in the medium, only about 5 mg of ferredoxin was isolated, whereas, flavodoxin constituted almost 2% of the soluble protein.

It is apparent that ferredoxin is replaced by flavodoxin in cells grown in low-iron media. In addition, Schönheit et al. (36) have shown that degradation of ferredoxin occurs in *C. pasteurianum* during periods of iron deprivation and it is utilized as a source of iron. Therefore, it is interesting that under low-iron conditions *C. formicoaceticum* and *M. elsdenii* (27) produce significant amounts of rubredoxin. This may indicate an important role for rubredoxin in these bacteria. However, the only postulated role so far for rubredoxin in anaerobic bacteria involves CO dehydrogenase, since rubredoxin is the most active acceptor of CO dehydrogenase electrons in the acetogenic bacteria (33, 34).

The molecular weights and other properties of ferredoxin, flavodoxin, and rubredoxin from *C. formicoaceticum* were similar to those reported for those proteins from other bacteria. By using amino acid analysis to determine the amount of protein and plasma emission spectroscopy as well as a colorimetric method to determine iron, we found that ferredoxin contained only four Fe and four S per mole of protein. The extinction coefficient, using the same absolute method for determination of protein concentration, agreed with the conclusion that the *C. formicoaceticum* ferredoxin

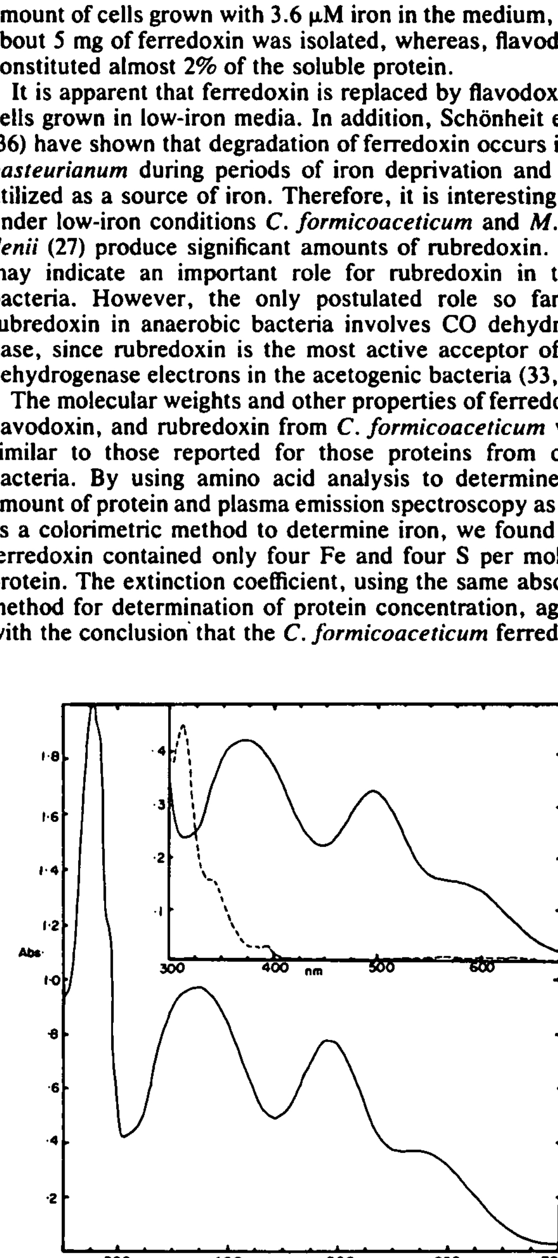

FIG. 2. Light absorption spectrum of *C. formicoaceticum* rubredoxin, 0.64 mg/ml in 50 mM Tris-hydrochloride, pH 7.6. Inset shows rubredoxin, 0.25 mg/ml, oxidized (———) and enzymatically reduced with *C. thermoaceticum* CO dehydrogenase (----) by bubbling with CO for 5 min before addition of enzyme.

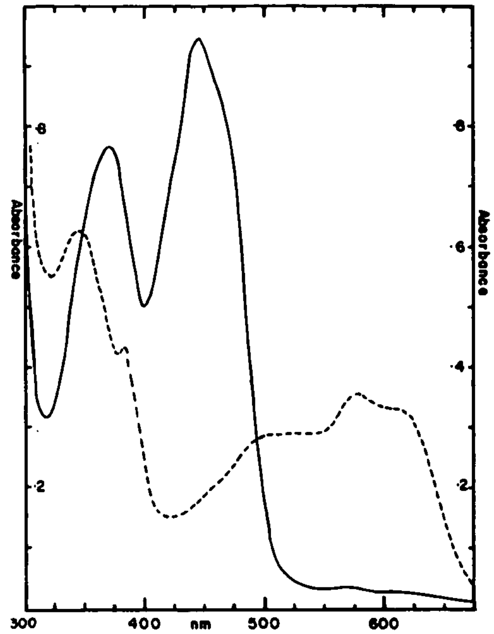

FIG. 3. Light absorption spectrum of *C. formicoaceticum* flavodoxin, 1.80 mg/ml, in 50 mM Tris-hydrochloride, pH 7.6. Oxidized flavodoxin (———) was enzymatically reduced with *C. thermoaceticum* CO dehydrogenase (----) by bubbling with CO for 5 min before addition of enzyme.

is a four-iron ferredoxin, like that of *C. thermoaceticum* ferredoxin I (44) and ferredoxins from *Desulfovibrio gigas* (39), *Desulfovibrio desulfuricans* (45), *Bacillus stearothermophilus* (31), *Spirochaeta stenostrepa* (18), and *Bacillus polymyxa* (37). However, the *C. formicoaceticum* ferredoxin contains eight cysteine residues, whereas *B. stearothermophilus* (15), Spirochaeta stenostrepa (18), and *B. polymyxa* (37) ferredoxins contain four and *C. thermoaceticum* (13), *D. gigas* (39), and *D. desulfuricans* (45) ferredoxins have six cysteine residues. Since all ferredoxins so far isolated which contain eight cysteines also contain eight Fe and eight S, it seems that the *C. formicoaceticum* ferredoxin should be convertible into an eight Fe-eight S protein. Even though the purification procedure utilized in preparation of the ferredoxin was quick and rather mild, it is possible that a cluster could have dissociated from the protein. Sequence analysis should explain the difference between this and other eight cysteine-containing ferredoxins.

The *C. formicoaceticum* rubredoxin light absorption peaks and the ratio of the peak at 490 nm to that at 280 nm were similar to those found for other purified rubredoxins. However, the *C. formicoaceticum* we found lacked a shoulder at approximately 350 nm that has been characteristic of other rubredoxins (10, 23, 43). In addition, the electron paramagnetic resonance spectrum (not shown here) of the *C. formicoaceticum* rubredoxin showed only the $g = 4.3$ resonance, and we did not detect the $g = 9.4$ resonance seen in other rubredoxins. This $g = 9.4$ signal is quite temperature sensitive and is always weaker than the $g = 4.3$ resonance, however, and electron paramagnetic resonance studies at various temperatures have not yet been carried out. We have not yet found the reasons for these spectral differences, since the iron and amino acid analyses were so similar to those of other rubredoxins.

Flavodoxin from *C. formicoaceticum* was similar to other flavodoxins (21, 27). When cells were grown in a medium containing 3.6 μM iron salts, it was a major component of the cell and constituted at least 2% of the soluble cell protein. The flavodoxin from *C. formicoaceticum* was used in resonance coherent and anti-Stokes Raman scattering spectroscopy and was found to resemble the *M. elsdenii* flavodoxin, in contrast to the *Desulfovibrio* and *Azotobacter vinelandii* flavodoxins (40).

Recently, ferredoxin, rubredoxin, and flavodoxin from *C. formicoaceticum* have been used as electron acceptors for *C. thermoaceticum* (33), *C. formicoaceticum*, and *Acetobacterium woodii* carbon monoxide dehydrogenases (34). We found in all cases that rubredoxin is the best natural electron acceptor, followed by ferredoxin and the flavodoxin. We postulate that rubredoxin, therefore, is the primary natural electron carrier for CO dehydrogenase in acetogenic bacteria.

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Purification and Characterization of Cytochrome c_3, Ferredoxin, and Rubredoxin Isolated from *Desulfovibrio desulfuricans* Norway

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Different electron carriers of the non-desulfoviridin-containing, sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Norway strain) have been studied. Two nonheme iron proteins, ferredoxin and rubredoxin, have been purified. This ferredoxin contains four atoms of non-heme iron and acid-labile sulfur and six residues of cysteine per molecule. Its amino acid composition suggests that it is homologous with the other *Desulfovibrio* ferredoxins. The rubredoxin is also an acidic protein of 6,000 molecular weight and contains one atom of iron and four cysteine residues per molecule. The amino acid composition and molecular weight of the cytochrome c_3 from *D. desulfuricans* (strain Norway 4) are reported. Its spectral properties are very similar to those of the other cytochromes c_3 (molecular weight, 13,000) of *Desulfovibrio* and show that it contains four hemes per molecule. This cytochrome has a very low redox potential and acts as a carrier in the coupling of hydrogenase and thiosulfate reductase in extracts of *Desulfovibrio gigas* and *Desulfovibrio desulfuricans* (Norway strain) in contrast to *D. gigas* cytochrome c_3 (molecular weight, 13,000). A comparison of the activities of the cytochrome c_3 (molecular weight, 13,000) of *D. gigas* and that of *D. desulfuricans* in this reaction suggests that these homologous proteins can have different specificity in the electron transfer chain of these bacteria.

The classification of sulfate-reducing bacteria within the genus *Desulfovibrio* is based upon several properties: absence of sporulation, desoxyribonucleic acid composition, growth on certain carbon sources, and, particularly, presence of desulfoviridin and cytochrome c_3 (molecular weight [MW], 13,000) (22).

The Norway strain of *Desulfovibrio desulfuricans* (19), although in most respects typically a *Desulfovibrio*, is characterized by an absence of desulfoviridin. It has recently been demonstrated by Jin-Po Lee et al. (14) that another pigment, desulforubidin, replaces desulfoviridin as a sulfite reductase for this organism. It was thus of taxonomic and biochemical interest to investigate the electron carrier content of the Norway strain to see if other differences could be found between the latter and other strains of *Desulfovibrio* such as *D. vulgaris*, strain Hildenborough, *D. gigas*, and *D. desulfuricans* strain El Agheila Z, all known to contain desulfoviridin as a sulfite reductase.

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In this work we report the purification from the Norway strain of several electron carrier proteins, together with some of their physicochemical properties and their reactivity toward thiosulfate reduction.

MATERIALS AND METHODS

Organism and growth conditions. *D. desulfuricans* (strain Norway 4, National Collection of Industrial Bacteria no. 8310) was grown in a lactate-sulfate medium and harvested as published previously (16).

Enzyme assays. A manometric assay was utilized for determination of the biological activity of the purified electron carriers utilizing the reduction of thiosulfate with H_2. The main compartment of each manometric vessel contained 0.1 M phosphate buffer (pH 7.0), the carrier, hydrogenase, and the reductase preparation in a final volume of 3.0 ml. A 15-μmol amount of sodium thiosulfate, freshly prepared, was added from a side arm after preincubation of the flask for 30 min under H_2 at 37°C. The center well contained 0.1 ml of 10% $CdCl_2$ and 0.1 ml of 10 N NaOH. Thiosulfate reductase activity was measured by following the initial rates of hydrogen utilization. The thiosulfate reductase-hydrogenase-

containing extracts, devoid of electron carriers, were prepared from *D. gigas* and *D. desulfuricans* (Norway strain) as already reported (11).

However, as the hydrogenase activity of *D. desulfuricans* appeared to be very sensitive to ammonium sulfate fractionation, pure hydrogenase prepared from *D. gigas* by the method of Bell (G.R. Bell, Ph.D. thesis, University of Georgia, Athens, 1973) and exhibiting a specific activity of 30 μmol of H_2 consumed/min per mg was added in all cases to the systems to insure an excess of this activity. Ferredoxin and cytochromes c_3 (MW 13,000) and c_3 (MW 26,000) from *D. gigas*, used to study the electron transfer between hydrogenase and thiosulfate reductase, were purified as described previously (4, 15, 16).

Amino acid analysis. Protein samples were hydrolyzed for 18, 20, and 48 h at 110°C in 6.0 M HCl in evacuated, sealed tubes according to the method of Moore and Stein (20). The average was calculated from at least five analyses. The amino acid composition was determined with a Beckman Multichrom amino acid analyzer. The values for serine and threonine have been corrected after extrapolation to zero hydrolysis time. Cysteine and methionine were analyzed after performic acid oxidation as cysteic acid and methionine sulfone, respectively, according to Hirs (12).

Molar extinction coefficients. The molar extinction coefficients of the proteins were obtained by measuring the values of the optical densities of their absorption maxima of a solution of known protein concentration. The molarity of the used solutions was calculated from amino acid analysis.

Iron determination. Non-heme (ferrous iron) was determined by the *o*-phenanthroline method of Harvey et al. (10), as modified by Lovenberg et al. (17). The absorption of the complex of the ferrous iron and *o*-phenanthroline was measured at 512 nm.

Inorganic sulfide determination. The inorganic sulfide content of ferredoxin was determined by using an adaptation of the method of Fogo and Popowski (8) by Lovenberg et al. (17) A 1.3-ml amount of 1% zinc acetate and 0.05 ml of 12% sodium hydroxide were added to the sample to be analyzed. A 0.25-ml portion of 0.5% *N,N*-dimethylenephenylenediamine hydrochloride and 0.05 ml of 0.023 M ferric chloride were then added to each tube. After 20 min, 0.85 ml of water was added, and the absorbance was determined at 670 nm (A_{670}). The tubes were stoppered and open only for the time necessary for each addition of reagents.

Molecular weight determination. The molecular weights were estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, using the procedure of Weber and Osborn (27), and by molecular filtration on a Sephadex column.

Spectral studies. The visible and ultraviolet spectra were determined with a Cary 14 spectrophotometer. A spectrophotometric method was also used for the monitoring of the purification of the electron carrier proteins, using a specific absorption band: 552 nm for cytochrome c_3, 390 nm for ferredoxin, and 490 nm for rubredoxin.

RESULTS

Protein purification. (i) Preparation of extracts. In these protein fractionation procedures, unless otherwise noted, all buffers were at pH 7.6 and all operations were performed at +4°C.

For preparation of the crude extract, 50 mM tris(hydroxymethyl)aminomethane (Tris)hydrochloride was added to 1,069 g (wet weight) of bacterial paste to bring the volume to 1,600 ml. After addition of a few deoxyribonuclease crystals, the cell suspension was passed twice through a Gaulin homogenizer. The resulting extract was centrifuged for 2 h at 39,000 × *g*, and the pellet was discarded.

(ii) Cytochromes. The clear extract was stirred overnight with 200 ml of silica gel, and the gel, to which the cytochromes were adsorbed, was separated by decantation. The decanted extract was saved for acidic protein purification (see below). The silica gel was washed several times with 10 volumes of 10 mM Tris-hydrochloride buffer until the supernatant was free of protein. The cytochromes were then eluted from the gel with 1 M K_2HPO_4, giving 650 ml of extract, which was dialyzed overnight against 10 liters of distilled water. The cytochromes were then adsorbed on a calcinated alumina column (4 by 14 cm) equilibrated with 10 mM Tris-hydrochloride buffer. The column was washed with 100 ml of the same buffer, and the protein was eluted with 1 M potassium phosphate buffer, giving 110 ml of extract.

After dialysis against 10 liters of distilled water, the cytochrome fraction was lyophilized, resuspended in 50 ml of 10 mM Tris-hydrochloride buffer, and placed on a Sephadex G-75 column (4 by 200 cm). Three cytochrome bands were separated: the first (with the largest molecular weight) was not purified further; the second contained cytochrome c_3; and the third (with the smallest molecular weight) contained a cytochrome presenting a "split α" band upon reduction with ascorbic acid. This cytochrome was called cytochrome $c_{553(550)}$ by analogy with the cytochrome found by Shioi et al. (24) in *Chloropseudomonas ethylica*.

(iii) Purification of cytochrome c_3. The cytochrome c_3 fraction from the G-75 column had a volume of 394 ml and a purity coefficient, defined as (A_{553} [red.] − A_{570} [red.])/A_{280} [ox.], of 3.03. The cytochrome was adsorbed on an Amberlite CG-50 type II column (4 by 15 cm) equilibrated with 10 mM Tris-hydrochloride buffer and eluted stepwise with 50 ml each of 50, 100, 200, 300, and 500 mM Tris-hydrochloride buffer. The cytochrome was eluted in a 110-ml fraction,

and its purity coefficient was 3.10. Finally, the cytochrome was filtered through a G-50 Sephadex column (5 by 200 cm) equilibrated with 10 mM Tris-hydrochloride buffer. The cytochrome-containing fraction had a volume of 360 ml, and the purity coefficient was 3.30, higher than any coefficient for cytochrome c_3 found so far. After dialysis against 20 liters of distilled water, the cytochrome was lyophilized. The yield was equal to 550 mg of cytochrome c_3, judged to be pure by polyacrylamide gel electrophoresis.

(iv) Purification of the non-heme iron proteins. A settled volume of diethylaminoethyl (DEAE)-cellulose, equal to 300 ml of DEAE-cellulose, was added to the extract obtained after the silica gel treatment used to remove the cytochromes, and the mixture was stirred overnight. The DEAE-cellulose was then decanted and washed several times with 10 mM Tris-hydrochloride buffer. The adsorbed acidic proteins were eluted with 1 M Tris-hydrochloride buffer, giving a volume of 480 ml. This extract was dialyzed against 10 liters of distilled water and placed on a DEAE-cellulose column (4 by 30 cm) equilibrated with 10 mM Tris-hydrochloride. The column was washed with 200 ml of 150 mM Tris-hydrochloride, and the protein was eluted with 1 M Tris-hydrochloride in a volume of 120 ml. The volume of the extract was brought to 1,200 ml by addition of distilled water, and the proteins were adsorbed on a second DEAE-cellulose column (4 by 25 cm) and eluted with a linear gradiant (2,000 ml) from 200 mM to 1 M Tris-hydrochloride. The first colored protein to be eluted was found to be rubredoxin, and two other bands of more acidic proteins had the typical absorption spectrum of ferredoxin. In contrast to all *Desulfovibrio* species we have studied so far, no flavodoxin band was found, and the ferredoxin content of the extract was clearly higher in the Norway strain than in the others.

(a) Purification of the rubredoxin. After the second DEAE-cellulose column, the rubredoxin was contained in a volume of 160 ml. The volume of the extract was brought to 350 ml with distilled water, and the rubredoxin was adsorbed on a DEAE-cellulose column (4 by 30 cm). The proteins were eluted with a linear gradiant (1,200 ml) of 250 to 500 mM Tris-hydrochloride, and the rubredoxin was collected in a volume of 220 ml. The extract was directly applied to a silica gel column (2.5 by 30 cm) equilibrated with 350 mM Tris-hydrochloride. The rubredoxin migrated slowly in the column and was eluted in a volume of 180 ml. It was then adsorbed on a calcinated alumina column (2.5 by 10 cm) equilibrated with 350 mM Tris-hydrochloride; the column was washed with 50 ml of the same buffer, and the rubredoxin was eluted with 10 mM Tris-hydrochloride in a volume of 30 ml. The protein was judged to be pure both from its spectrum (A_{280}/A_{490} = 2.35, identical to other rubredoxins from desulfovibriones) and from polyacrylamide gel analysis; it was then dialyzed against 10 liters of distilled water and lyophilized, giving a yield of 45 mg. Some of its spectral characteristics are reported in Table 1.

(b) Purification of ferredoxin. The ferredoxin is acidic and was eluted with 500 mM Tris-hydrochloride (in a volume of 175 ml) from the second DEAE-cellulose column. The ferredoxin fraction was placed on a calcinated alumina column (5 by 25 cm), equilibrated with 500 mM Tris-hydrochloride, in order to remove a contaminant with a strong absorption at 260 nm. The ferredoxin was collected in the same buffer in a volume of 170 ml, and the volume was brought to 250 ml with distilled water. The ferredoxin was then adsorbed on a DEAE-cellulose column (4 by 25 cm) and eluted with a linear gradiant (800 ml) from 350 to 550 mM Tris-hydrochloride. It was collected in a volume of 200 ml and placed on a calcinated alumina column (2 by 15 cm), equilibrated with 500 mM Tris-hydrochloride, in order to remove the last traces of the 260-nm contaminant, and was collected in a volume of 195 ml. The volume was brought to 240 ml with distilled water, and the ferredoxin was adsorbed on a DEAE-Sephadex column (2 by 25 cm) equilibrated with 400 mM Tris-hydrochloride. The protein was eluted with a linear gradiant (300 ml) from 400 to 600 mM Tris-hydrochloride and collected in a volume of 74 ml. It was dialyzed against 2 liters of

TABLE 1. *Molar extinction coefficients of rubredoxin, ferredoxin, and cytochrome c_3 from D. desulfuricans Norway*

Protein[a]	λ/nm	ε/mol per cm
Rubredoxin (ox.)	490	6,900
	375.5	8,142
	279	15,870
Ferredoxin (ox.)	390	17,500
	305	20,588
Cytochrome c_3 (ox.)	531.5	50,132
	408.5	530,918
	351	118,737
Cytochrome c_3 (red.)	552	128,481
	523	662,46
	418.5	891,944

[a] ox., Oxidized; red., reduced.

distilled water, concentrated, and precipitated by dialysis against 500 ml of saturated ammonium sulfate solution. The precipitate was collected by centrifugation, washed in saturated ammonium sulfate, and finally resuspended in 10 ml of the same solution. The precipitate was dissolved by the addition of a few drops of distilled water, and a few crystals of ammonium sulfate were added to the solution. Crystals were formed in a few hours. This crystallization process was repeated twice. A small fraction of the crystallized ferredoxin was then dialyzed against distilled water, and the protein was judged to be pure from its spectrum (ratio A_{305}/A_{390} = 0.84, identical to ferredoxins from other desulfovibriones) and by polyacrylamide gel analysis. The ferredoxin was usually kept in a crystalline form in ammonium sulfate solution and dialyzed against distilled water just prior to its utilization. The yield was 55 mg of pure ferredoxin.

The absorption spectrum of ferredoxin shows two peaks, one at 305 nm and the other at 390 nm, with a shoulder at 290 nm. The spectral properties are similar to those of the *D. gigas* ferredoxin (15). The molar extinction coefficient is 17,500 at 390 nm (see Table 1).

(v) Coupling activity of different electron carriers between hydrogenase and thiosulfate reductase. The coupling activities between hydrogenase and thiosulfate reductase of cytochrome c_3 and ferredoxin from *D. desulfuricans* and *D. gigas* were tested using reductase preparations devoid of electron carriers from both microorganisms. The concentrations were chosen to give maximal activity. In addition, the activity of *D. gigas* cytochrome c_3 (MW 26,000) in this reaction was compared with that obtained with the above-mentioned electron carriers. Cytochrome c_3 from *D. desulfuricans* is an efficient carrier in this reaction with enzymatic preparation from the same organism, whereas *D. gigas* cytochrome c_3 is inactive with the *D. desulfuricans* thiosulfate reductase and with *D. gigas* gives only a slight stimulation of thiosulfate reductase independent of the concentration, as already reported (11) (Fig. 1 and 2). The activity exhibited by cytochrome c_3 from *D. desulfuricans* increases with increased cytochrome concentrations, and the maximum activity is reached with 24 nmol of cytochromes, using the same amount of reductase as in the experiment reported in Fig. 1. Both ferredoxins from *D. desulfuricans* and *Desulfovibrio gigas* are carriers in the coupling between hydrogenase and thiosulfate reductase from *D. gigas*; however, the two ferredoxins are less efficient with the enzymatic preparation from *D. desulfuricans*. Finally, as already reported (4), cytochrome c_3 (MW 26,000) from *D. gigas* gives a strong stimulation with both enzymatic preparations of the same magnitude, on a molar basis, as that obtained with methyl viologen.

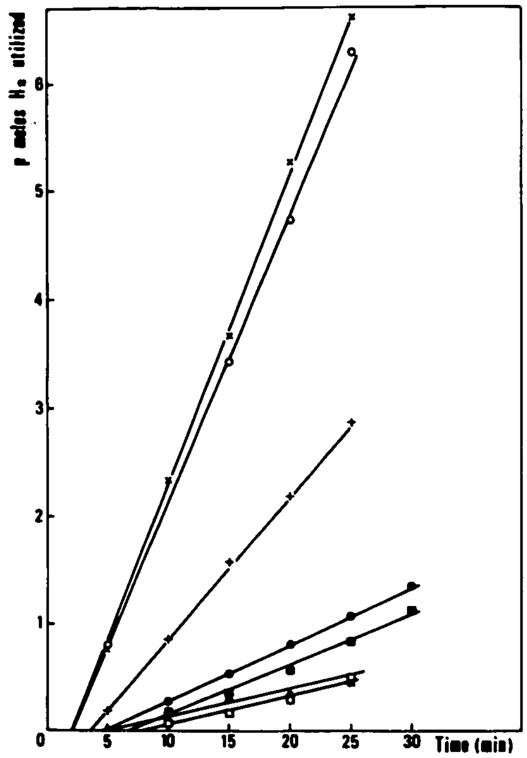

FIG. 1. *Reduction of thiosulfate by H_2 in the presence of various electron carriers with D. desulfuricans enzymatic preparation. The reaction mixture contained all the reactants indicated in Materials and Methods. Each flask contained enzymatic preparation (31 mg) and hydrogenase (400 µg). Symbols: (▲) control without added electron carrier; (○) plus methyl viologen (100 nmol); plus D. desulfuricans cytochrome c_3 (20 nmol); (●) plus D. desulfuricans ferredoxin (100 nmol); plus D. gigas cytochrome c_3 (MW 13,000) (20 nmol); (■) plus D. gigas ferredoxin (100 nmol); (×) plus D. gigas cytochrome c_3 (MW 26,000) (10 nmol).*

Amino acid compositions. The amino acid values were determined from the average of several acid hydrolysates. Corrections were made, when necessary, for the degradation or slow release of certain residues. The number of residues per mole of protein was determined by the mole ratio method.

(i) Ferredoxin. The amino acid composition and iron sulfide content of *D. desulfuricans* Norway ferredoxin are presented in Table 2. The protein contains 54 amino acids and is

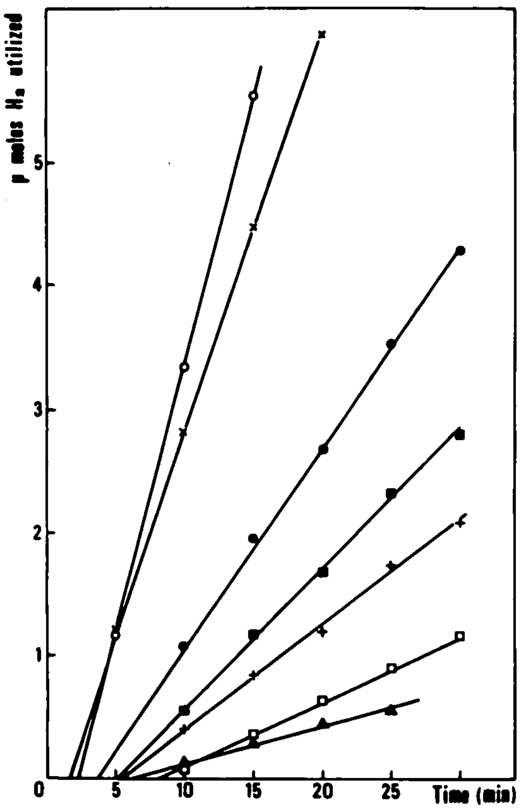

FIG. 2. *Reduction of thiosulfate by H_2 in the presence of various electron carriers with D. gigas enzymatic preparation. The reaction mixture contained all the reactants indicated in Materials and Methods. Each flask contained enzymatic preparation (22 mg of protein) and hydrogenase (400 μg). Symbols:* (▲) *control without added electron carrier;* (○) *plus methyl viologen (100 nmol);* (+) *plus D. desulfuricans cytochrome c_3 (30 nmol);* (●) *plus D. desulfuricans ferredoxin (100 nmol;* (□) *plus D. gigas cytochrome c_3 (MW 13,000) (30 nmol);* (■) *plus D. gigas ferredoxin (100 nmol);* (×) *plus cytochrome c_3 (MW 26,000) (10 nmol).*

devoid of histidine and tyrosine, like *D. gigas* ferredoxin (25). The minimum calculated molecular weight, including iron and sulfide, is 6,134. This value is in good agreement with the value estimated by polyacrylamide gel electrophoresis with sodium dodecyl sulfate, by reference with the migration of *D. gigas* ferredoxin (MW 6,056).

The analytical results show the presence of four iron atoms, four sulfide groups, and six cysteine residues.

The amino acid composition of the *Desulfovibrio* ferredoxins *D. gigas* and *D. desulfuricans* Berre S (29) show identical contents of half-cystine, labile sulfur, and iron. All the *Desulfovibrio* ferredoxins have a low content of basic amino acid residues and aromatic residues.

(ii) **Rubredoxin.** Hydrolysis of several samples of rubredoxin with 6 N hydrochloric acid under vacuum at 110°C for 20 h, followed by quantitative amino acid analysis, showed the amino acid content listed in Table 3. The amino acid compositions of the three *Desulfovibrio* rubredoxins so far reported are added for comparison. Acidic amino acids are preponderant, and all the rubredoxins are devoid of histidine and arginine residues, which is consistent with chromatographic properties on DEAE-cellulose. All the rubredoxins contain four cysteine residues, one non-heme iron, and no acid-labile sulfide. Calculation of molecular weight from the amino acid composition gave a result of 6,728, including one atom of iron per molecule.

(iii) **Cytochrome c_3.** Cytochrome c_3, first described by Postgate (22), is characteristic of the genus *Desulfovibrio*. It was then proposed that it contains two hemes per molecule, but from amino acid sequence analysis (1, 3; R. P. Ambler, Biochem. J. 109:47P-48P) it became clear that cytochrome c_3 possesses four binding clusters for the hemes. Recent results of Yagi and Maruyama (28), Meyer et al. (18), and Dobson et al. (6) are consistent with the presence of four hemes per molecule. The amino acid composition of *D. desulfuricans* strain Norway cytochrome c_3 is presented in Table 4 and compared with that obtained for cytochromes c_3 from *D. vulgaris* strain Hildenborough, *D. gigas*, and *D. desulfuricans* strain El Agheila Z. The main characteristics of all cytochromes c_3 are a high number of cysteine residues (8), of histidine residues (6-10) and of lysine residues.

After filtration on a G-50 Sephadex column, a molecular weight of 16,000 was determined. This figure is higher that the value of 13,000 determined for other c_3-type cytochromes and renders difficult a nomenclature of this type of hemoprotein based on molecular weight. The molecular weight based on the amino acid composition is 16,096. These values are consistent and give an average molecular weight for *D. desulfuricans* strain Norway cytochrome c_3 of 16,000.

DISCUSSION

The non-heme iron proteins of *D. desulfuricans*, Norway strain, do not differ in molecular weight, iron, and acid-labile sulfur content or in the number of cysteine residues from the other characterized Desulfovibrio ferredoxins and rubredoxins. Like the other bacterial rubredoxins, the rubredoxin is devoid of histidine and arginine residues and possesses four cys-

TABLE 2. *Amino acid composition of D. desulfuricans Norway ferredoxin*[a] *and comparison with other Desulfovibrio sp.*

Amino acid	Amino acid residues/molecule			
	D. desulfuricans (Norway) ferredoxin		Ferredoxin	
	From analysis	Nearest integer	*D. gigas*[b]	*D. desulfuricans* Berre S[c]
Lysine	1.8	2	1	2
Histidine	0	0	0	1
Ammonia				
Arginine	0	0	1	0
Tryptophan	ND	ND	0	0
Aspartic acid	4.8	5	11	10
Threonine	2.6	3	0	3
Serine	2.5	3	3	2
Glutamic acid	11	11	9	11
Proline	2.6	3	4	3
Glycine	2	2	1	6
Alanine	6.8	7	6	2
Cystine (half)[d]	6	6	6	6
Valine	3	3	5	5
Methionine[d]	1.9	2	2	0
Isoleucine	4.8	5	5	4
Leucine	1	1	1	2
Tyrosine	0	0	0	0
Phenylalanine	0.8	1	1	0
Total residues		54	56	57
Non-heme iron[e]		4	4	4
Acid-labile sulfide[e]		4	4	4

[a] Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum. Abbreviation: ND, Not determined.
[b] From Travis et al. (25).
[c] From Zubieta et al. (29).
[d] Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).
[e] Atoms per molecule.

teine residues, one non-heme iron, and no acid-labile sulfide. The ferredoxin is devoid of histidine and tyrosine like *D. gigas* ferredoxin and has four iron atoms, four sulfide groups, and six cysteine residues. It is noteworthy that two ferredoxins were detected in the Norway strain. This second ferredoxin is more acidic and can be separated from the other on a DEAE-cellulose column. We have already noted that, by comparison with other *Desulfovibrio* species, ferredoxin is more abundant, and this seemed to be related to the absence of flavodoxin in the Norway strain. However, during the purification, it became clear that the second ferredoxin was quite unstable. As a result, only small amounts of this ferredoxin were recovered in a pure state, just enough for purity tests and amino acid determination. No reactivity experiments could be performed.

In earlier publications (2, 5), we have reported the presence of three *c*-type cytochromes in *D. vulgaris* and *D. desulfuricans* (El Agheila Z), namely, c_{553}, c_3 (MW 13,000), and c_3 (MW 26,000). The latter contain eight hemes per molecule; an analogous cytochrome was not detected in the Norway 4 strain.

The data on the coupling activity of *D. desulfuricans* (Norway 4) cytochrome c_3, ferredoxin, and the homologous electron carriers of *D. gigas* between hydrogenase and thiosulfate reductase indicate that *D. desulfuricans* (Norway 4) cytochrome c_3 gives only a slight stimulation with the *D. gigas* reductase, as already reported (5). On the other hand, the ferredoxins of the two bacteria function as carriers in this reaction; however, these proteins are weakly active with the reductase preparation of *D. desulfuricans* (Norway 4). As described earlier (11), cytochrome c_3 (MW 26,000) from *D. gigas* appears to be the most efficient carrier in the

TABLE 3. *Amino acid composition of D. desulfuricans Norway rubredoxin*[a] *and comparison with other Desulfovibrio rubredoxins*

Amino acid	*D. desulfuricans* (Norway) rubredoxin		Rubredoxin		
	From analysis	Nearest integer	*D. gigas*[b]	*D. desulfuricans azotovorans*[c]	*D. vulgaris*[d]
Lysine	4.8	5	5	4	5
Histidine	0	0	0	0	0
Ammonia					
Arginine	0	0	0	0	0
Tryptophan	ND	ND	1	1	1
Aspartic acid	12.6	13	8	7	9
Threonine	3.5	4	2	2	2
Serine	0	0	4	2	4
Glutamic acid	5.2	5	5	8	5
Proline	5.1	5	5	6–7	7
Glycine	7.2	7	6	6	7
Alanine	5.0	5	5	6	6
Cystine (half)[e]	4	4	4	4	4
Valine	6.0	6	4	5	5
Methionine	0.82	1	1	1	1
Isoleucine	0	0	3	2	0
Leucine	0.94	1	2	0	1
Tyrosine	4.1	4	3	3	2
Phenylalanine	2.1	2	3	3	2
Total residues		63	61	60	61
Non-heme iron[f]		1	1	1	1
Acid-labile sulfide[f]		0	0	0	0

[a] Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum. ND, Not determined.

[b] From Laishley et al. (13).

[c] From Newman and Postgate (21).

[d] From Bruschi and Le Gall (4).

[e] Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).

[f] Atoms per molecule.

coupling of hydrogenase with thiosulfate reductase. It should be emphasized that with our enzymatic preparation, devoid of electron carriers but containing thiosulfate and sulfite reductase activities, we used excess thiosulfate as substrate and measured only the reduction of thiosulfate, since sulfite is used only when all the thiosulfate has disappeared (11).

Wagner et al. (26) have reported the standard potential of thiosulfate reduction to sulfide and sulfite to be near −420 mV. This observation suggests that only a low-potential electron carrier could couple hydrogenase and thiosulfate reductase. The redox potential of *D. desulfuricans* cytochrome c_3 has been found to be in the vicinity of −500 mV (P. Bianco, personnal communication), a high electronegative value as compared with the potential (−250 mV) reported for *D. gigas* cytochrome c_3 (MW 13,000) (16). Thus, in *D. desulfuricans* (Norway 4), cytochrome c_3 is a good candidate for transferring electrons during thiosulfate reduction. However, in addition to the requirement for a low redox potential, the specificity of the electron carrier for hydrogenase and thiosulfate reductase is an important factor since ferredoxins from both bacteria, although extremely electronegative, give only a small stimulation at a concentration of 33.3 μM with the *D. desulfuricans* (Norway 4) reductase preparation. With this latter preparation the efficiency of *D. desulfuricans* (Norway 4) cytochrome c_3 in the coupling of hydrogenase with thiosulfate reductase is near that of *D. gigas* cytochrome c_3 (MW 26,000). In this case, a concentration of *D. desulfuricans* (Norway 4) cytochrome c_3 twice that of *D. gigas* cytochrome c_3 (MW 26,000) was used since, as already reported (5), the former cytochrome contains four hemes per molecule and the latter contains eight hemes. Then, in

TABLE 4. *Amino acid composition of D. desulfuricans Norway cytochrome c_3 (MW 13,000)*[a] *and comparison with other Desulfovibrio cytochromes*

Amino acid	*D. desulfuricans* (Norway) cytochrome c_3		Cytochrome c_3 (MW 13,000)		
	From analysis	Nearest integer	*D. vulgaris*[b]	*D. gigas*[c]	*D. desulfuricans* El Agheila Z[d]
Lysine	15.99	16	20	17	15
Histidine	7.2	7–8	9	8	8
Ammonia					
Arginine	1.9	2	1	0	1
Tryptophan	0	0	0	1	1
Aspartic acid	15.1	15	12	18	8
Threonine	9.7	10	5	5	5
Serine	5.6	6	6	6	8
Glutamic acid	10.1	10	5	4	6
Proline	7.8	8	4	4	6
Glycine	9.7	10	9	11	8
Alanine	12.8	13	10	9	13
Cystine (half)[e]	8	8	8	8	8
Valine	6.0	6	8	8	5
Methionine	0.8	1	3	0	4
Isoleucine	3.6	4	0	4	2
Leucine	4.8	5	2	42	0
Tyrosine	1.2	2	3	2	1
Phenylalanine	3.8	4	2		3
				111	
Total residues		127	107		102

[a] Amino acid analyses were carried out using general methods with 20-h hydrolysis (6 N HCl) at 110°C under vacuum.

[b] From Ambler (Biochem. J. 109:47P–48P, 1968).

[c] From Ambler et al. (1).

[d] From Ambler et al. (3).

[e] Cysteine was determined as cysteic acid after performate oxidation of the protein sample according to the method of Hirs (12).

D. desulfuricans (Norway 4), cytochrome c_3 replaces cytochrome c_3 (MW 26,000) as an electron carrier in the electron transport system of thiosulfate reduction. These observations suggest that cytochromes c_3, although homologous proteins, have different physiological roles in the sulfate-reducing bacteria, since ferredoxin is not essential for coupling between hydrogenase and thiosulfate reductase in *D. desulfuricans* (Norway 4). In *D. gigas*, ferredoxin, flavodoxin, or cytochrome c_3 (MW 26,000) is a carrier in the same reaction, when cytochrome c_3 (MW 13,000) is almost inactive (11).

It can then be postulated that an important structural modification took place in *D. desulfuricans* (Norway 4) cytochrome c_3, allowing the disappearance of flavodoxin and the exclusion of ferredoxin from the electron transfer chain between hydrogenase and thiosulfate reductase so that the ferredoxin could be utilized in some other part of the complex electron transfer system typical of the sulfate-reducing bacteria. Comparison of the primary structure of other cytochromes c_3 (3) shows that only 25% of the total residues remain unchanged. Such a variability is not common in cytochromes of the *c* type and supports the hypothesis of changes in the function of cytochromes c_3 in sulfate-reducing bacteria. The higher molecular weight of *D. desulfuricans* (Norway 4) cytochrome c_3 is also in favor of this change. Both primary and tertiary structure determinations, which are now in progress (6, 9), will provide more precise information concerning the structure/function relationships in this class of hemoproteins.

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Analysis of the Transcriptional Unit Encoding the Genes for Rubredoxin (*rub*) and a Putative Rubredoxin Oxidoreductase (*rbo*) in *Desulfovibrio vulgaris* Hildenborough

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The nucleotide sequence of a 2.0-kilobase-pair *Eco*RI restriction fragment upstream from the gene (*rub*, 162 base pairs) encoding rubredoxin from *Desulfovibrio vulgaris* Hildenborough indicates that it is part of a larger transcriptional unit, containing an additional 378-base-pair open reading frame which terminates 16 nucleotides from the translational start of the *rub* gene and could encode a polypeptide of 14 kilodaltons (kDa). Northern (RNA) blotting of RNA isolated from both *D. vulgaris* Hildenborough and *Escherichia coli* TG2 transformed with plasmid pJK29, which contains both genes on a 1.1-kilobase-pair *Sal*I insert, confirms that the genes for this 14-kDa polypeptide and rubredoxin are present on a single transcript of 680 nucleotides. Strong evidence that the 14-kDa polypeptide is also a redox protein is provided by the fact that its NH_2 terminus is homologous to desulforedoxin, which has been isolated from *D. gigas* as a small dimeric redox protein (36 amino acids per monomer), coordinating two iron atoms. Since rubredoxin is a potential redox partner for the 14-kDa protein, it has been tentatively named rubredoxin oxidoreductase, produced by the *rbo* gene. Southern blotting indicates that the *rbo-rub* operon is present in several species and strains of sulfate-reducing bacteria.

Rubredoxins are small, electron-carrying proteins (molecular mass, 6 kilodaltons [kDa]), which are found in the cytoplasm of several anaerobic bacteria, e.g., *Desulfovibrio gigas* (8), *D. vulgaris* Hildenborough (7), *D. desulfuricans* (15), *Thermodesulfobacterium commune* (24), *Clostridium pasteurianum* (36), *Peptostreptococcus elsdenii* (4), and *Peptococcus aerogenes* (3). The amino acid sequences of these rubredoxins have been determined (3, 4, 7, 8, 15, 36), and the three-dimensional structures of rubredoxins from *C. pasteurianum*, *D. vulgaris*, *D. gigas*, and *D. desulfuricans*, which has a very low molecular mass (only 5.2 kDa) have been solved (2, 13, 14, 32, 41–43). The redox center of rubredoxin consists of a single iron atom (redox potential, −50 to 0 mV), coordinated to four cysteinyl sulfurs. This redox potential is relatively high, since dissimilatory sulfate reduction by *Desulfovibrio* species requires electrons at −400 to −200 mV (17, 18, 23, 27), and it is not clear, therefore, which electron transfer reaction is catalyzed by rubredoxin. Several attempts have been made to define the redox partners of rubredoxin by biochemical experiments. By using the rubredoxin-mediated reduction of eucaryotic cytochrome *c* by NADH as an assay, an NADH-rubredoxin oxidoreductase was (partially) purified from *D. gigas* (16) and *C. acetobutylicum* (25). The *Clostridium* enzyme was found to have flavin adenine dinucleotide as the prosthetic group and to consist of a single subunit with a molecular mass of 41 kDa (25). The *D. gigas* enzyme shows specificity for rubredoxin from *D. gigas* ($K_m = 6.2 \times 10^{-6}$ M) relative to rubredoxins from *D. vulgaris* ($K_m = 5.3 \times 10^{-5}$ M) and *C. pasteurianum* ($K_m = 1.0 \times 10^{-4}$ M), which show 71 and 63% sequence identity, respectively, with *D. gigas* rubredoxin. The subunit molecular mass and nature of the cofactor have not been reported for the *D. gigas* enzyme, and it has not been isolated from *D. vulgaris*.

A different approach to the identification of possible redox partners for rubredoxin, which makes use of the fact that the gene encoding rubredoxin (*rub*) from *D. vulgaris* Hildenborough was recently cloned from a λ library of the *D. vulgaris* chromosome and its nucleotide sequence was determined (37), is taken here. No typical promoter consensus sequence (29) was found in the region immediately upstream from the *rub* gene, which instead appears to contain the 3′ end of another reading frame, indicating that the *rub* gene may be part of a larger transcriptional unit. Since at least one gene encoding a redox protein interacting with rubredoxin could be present on this larger transcript, the region upstream from the *rub* gene is characterized in detail in the present paper.

MATERIALS AND METHODS

Strains, vectors, and media. The bacterial strains, plasmids, and cloning vectors used in this work are described in Table 1. For the isolation of RNA, *D. vulgaris* Hildenborough was grown anaerobically in a medium made up from the following solutions, described by Pfennig et al. (26): solution 1 (1 liter, kept under 90% N_2–10% CO_2), solution 2 (1 ml), solution 3 (1 ml), solution 4 (30 ml), solution 5 (3 ml), 15% (wt/vol) of sodium lactate (10 ml), solution 8 (1 ml), solution 9 (0.1 ml), and Wolin vitamins (10 ml) (44). This medium allows a high growth rate of *D. vulgaris*, and RNA can successfully be isolated from cells grown for 16 h at 30°C at a 5% (vol/vol) inoculum. Chromosomal DNA, isolated from 15 different species and strains of sulfate-reducing bacteria (see Table 2) after growth on Postgate medium C (27), was a gift from Helen Kent, AFRC Unit of Nitrogen Fixation, University of Sussex, Brighton, England. DNA from *D. vulgaris* Miyazaki was a gift from T. Yagi, Department of Chemistry, Shizuoka University, Shizuoka, Japan, and DNA from *D. salexigens* NCIMB 8365 was donated by D. W. S. Westlake, Department of Microbiology, University of Alberta, Edmonton, Canada. *Escherichia coli* TG2 was grown in TY medium (22) containing 10 g of tryptone, 5

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TABLE 1. Bacterial strains and DNA vectors used in this study

Strain or vector	Genotype, phenotype, comments, reference
Strains	
D. vulgaris subsp. *vulgaris* Hildenborough[a]	NCIMB 8303; isolated from clay soil near Hildenborough, U.K. (27)
E. coli TG2[b]	Δ(*lac-pro*) *supE thi hsdM hsdR recA* F' (*traD36 proAB*$^+$ *lacZ*ΔM15*I*^{q}); from T. J. Gibson.
Vectors	
pUC8, pUC9	Amp^r (35)
pJK15	This study (Fig. 1)
pJK29	This study (Fig. 1)
pRbo1	This study (Fig. 1)
pRub1	This study (Fig. 1)

[a] Abbreviated as *D. vulgaris* Hildenborough. A bacteriophage λ gene library has been constructed for this organism (37).
[b] Constructed from *E. coli* JM101 [Δ(*lac-pro*) *supE thi* F' (*traD36proAB*$^+$ *lacZ*ΔM15 *I*^{q}] by T. J. Gibson and M. D. Biggin, Laboratory of Molecular Biology, Medical Research Council Centre, Cambridge, U.K.

g of yeast extract, and 5 g of NaCl per liter of water at pH 7.4.

Biochemical reagents. All enzymes were obtained from Pharmacia, Inc., with the exception of calf alkaline phosphatase, which was from Boehringer Mannheim Biochemicals. The radioisotopes [α-^{35}S]dATP (400 Ci/mmol; 10 mCi/ml), [α-^{32}P]dATP (3,000 Ci/mmol; 10 mCi/ml), and [γ-^{32}P]ATP (3,000 Ci/mmol; 10 mCi/ml) were purchased from Amersham Corp. and were used for dideoxynucleotide sequencing, nick translation, and 3' and 5' end labeling, respectively. Ficoll 400 was purchased from Pharmacia, Inc. Polyvinylpyrrolidone (molecular weight 40,000), bovine serum albumin (fraction V), molecular biology grade sodium dodecyl sulfate (SDS), bakers' yeast tRNA, salmon sperm DNA (sodium salt), and dextran sulfate (molecular weight 500,000) were purchased from Sigma Chemical Co. Low- and high-gelling-temperature (LGT and HGT) agarose were obtained from Bethesda Research Laboratories, Inc. Nitrocellulose and Hybond-N hybridization transfer membranes were obtained from Schleicher & Schuell, Inc., and Amersham, respectively. All other reagent grade chemicals were purchased from either Sigma or Fisher Scientific Co.

DNA cloning. Several recombinant bacteriophages carrying the *rub* gene were isolated from a λ library, as described previously (37). These clones cover 35 kilobase pairs (kb) of the *D. vulgaris* Hildenborough chromosome, and an *Eco*RI restriction map of a part of this region is shown in Fig. 1. A 2.0-kb *Eco*RI fragment and a 1.1-kb *Sal*I fragment (Fig. 1) were cloned into the *Eco*RI and *Sal*I sites of pUC8 (35), generating plasmids pJK15 and pJK29, respectively, which were purified as described elsewhere (40).

Shotgun nucleotide sequencing. The procedure followed for shotgun nucleotide sequencing was essentially that outlined by Bankier and Barrell (5). Plasmid pJK15 was sonicated, and the resulting fragments were end repaired and size fractionated by gel electrophoresis on 1% (wt/vol) LGT agarose. The 400- to 1,000-base-pair (bp) fraction was excised and isolated from the gel and ligated to the replicative form of M13mp8 (21), digested with *Sma*I and calf alkaline phosphatase (5). After being transfected into competent, $CaCl_2$-treated *E. coli* TG2 cells, the ligation mixtures were spread onto TY plates with top agar, containing isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (5). Single-stranded DNA was isolated from white recombinant phage plaques by using a 1.5-ml minipreparation procedure (5). The purified DNAs (2 μl) were spotted on a nitrocellulose filter, which was subsequently baked at 80°C for 1 h under vacuum. The 2.0-kb *Eco*RI insert from pJK15 was isolated by LGT agarose gel electrophoresis, radiolabeled by nick translation

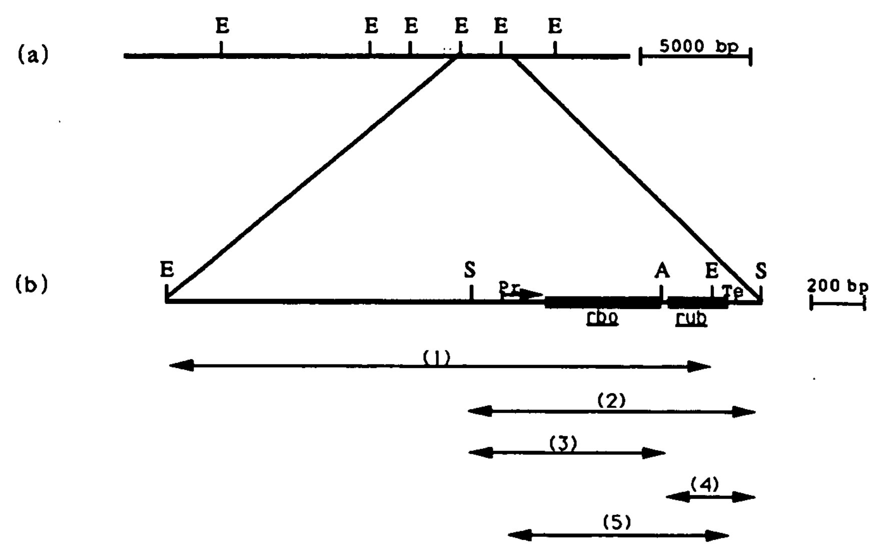

FIG. 1. Survey of DNA containing the *rub* gene of *D. vulgaris* Hildenborough. (a) Location of *Eco*RI (E) restriction sites as determined earlier (37). (b) Map of a 2,234-bp *Eco*RI-*Sal*I region of DNA for which the nucleic acid sequence was determined. The location of restriction sites for *Eco*RI, *Sal*I (S), and *Ava*I (A), the coding regions for the genes encoding rubredoxin (*rub*) and the putative rubredoxin oxidoreductase (*rbo*), and the location of promoter (Pr) and transcription terminator (Te) sequences, are indicated. The following plasmids (inserts) were constructed: 1, pJK15 (2.0 kb EE); 2, pJK29 (1.1 kb SS); 3, pRbo1 (746 bp AS) and 4, pRub1 (347 bp AS). The position of the nucleic acid sequence shown in Fig. 3 is indicated in region 5.

(20), and denatured by boiling. The filter was prehybridized in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.2])–10× Denhardt solution (20)–0.5% (wt/vol) SDS for 15 min at 68°C. The 2.0-kb DNA probe was then added to the prehybridization solution, and hybridization was continued for 16 h at 68°C. After hybridization, the filter was washed in 6× SSC–0.5% (wt/vol) SDS at 68°C for 1 h. The filter was then dried and wrapped in Saran wrap, and positive clones were identified by autoradiography. These were then sequenced by the dideoxy-chain termination procedure developed by Sanger et al. (31), as detailed by Bankier and Barrell (5). The sequencing data were processed and analyzed by using the programs of Staden and McLachlan (33, 34).

Construction of gene-specific probes. The completed nucleotide sequence indicated the presence of a unique *Ava*I site in the 19-bp region separating the *rbo* and *rub* genes (Fig. 1; see also Fig. 3). Plasmid pJK29 was digested with *Ava*I, and the digested DNA was end repaired with the Klenow fragment of DNA polymerase I to generate blunt ends (20). Following inactivation of the polymerase at 68°C, pJK29 was further digested with *Sal*I, resulting in the excision of both the *rbo* and *rub* genes on 746- and 347-bp fragments of DNA, respectively; these were isolated by electrophoresis on 1.5% (wt/vol) LGT agarose. The purified fragments were ligated into pUC8, previously digested with *Sal*I and *Sma*I, to give plasmids pRbo1 and pRub1, respectively. The insert of pRbo1 was gel isolated following digestion with *Hin*dIII and *Eco*RI as a 767-bp *rbo* gene-specific probe, whereas the presence of an *Eco*RI site near the 3′ end of the *rub* gene (Fig. 1; see also Fig. 3) allowed the isolation of a 162-bp *rub* gene-specific probe by digestion of pRub1 with *Eco*RI and gel electrophoresis.

RNA isolation and Northern blotting. RNA was isolated from cultures of *D. vulgaris* Hildenborough and *E. coli* TG2 by the hot-phenol extraction method (11) and stored as the ethanol precipitate at −20°C. Samples of RNA were collected by centrifugation, washed with 70% (vol/vol) ethanol, dried under vacuum, and redissolved in 20 μl of loading buffer, prepared as described elsewhere (10). For Northern (RNA) blotting, RNA samples were electrophoresed on gels containing 1.0% (wt/vol) HGT agarose in 1× MOPS buffer (10× MOPS buffer is 0.2 M 3-*N*-morpholinopropanesulfonic acid, 10 mM EDTA, and 50 mM sodium acetate [pH 7.0]) containing 2% (vol/vol) formaldehyde and then blotted onto a Hybond-N hybridization transfer membrane. Following transfer, the membrane was exposed to shortwave UV light to cross-link the RNA to the filter. Blots were prehybridized for 4 h at 42°C in a solution containing 0.75 M NaCl, 0.075 M sodium citrate (pH 7.0), 50% (vol/vol) formamide, 1% (wt/vol) SDS, 0.1% (wt/vol) Ficoll 400, 0.1% (wt/vol) polyvinylpyrrolidone, 0.1% (wt/vol) bovine serum albumin, and 0.16 mg of boiled bakers' yeast tRNA per ml. Following prehybridization, the blots were hybridized at 42°C for 16 h in the solution described above, also containing 10% (wt/vol) dextran sulfate as a hybridization enhancer, with a nick-translated probe derived from either plasmid pRbo1 or pRub1. Following hybridization, the blots were washed twice for 5 min at room temperature with 100 ml of 0.3 M NaCl–0.06 M Tris hydrochloride (pH 8.0)–0.002 M EDTA, then twice for 15 min at 60°C with 100 ml of the same solution with 0.5% (wt/vol) of SDS added, and, finally, twice for 15 min at room temperature with 0.003 M Tris base, after which the filters were dried and autoradiographed.

S1 nuclease mapping. A probe covering the region upstream from the *rbo* gene was prepared by digestion of pJK29 with *Eco*RI and calf alkaline phosphatase and gel isolation of the 913-bp *Eco*RI fragment, containing the relevant *Sal*I-*Eco*RI fragment. The probe was 5′ end labeled by adding T4 polynucleotide kinase (1 μl; 10 U/μl) and [γ-^{32}P]ATP (2.5 μl; 10 μCi/μl) in the presence of 50 mM Tris hydrochloride (pH 7.6)–10 mM $MgCl_2$–3 mM dithiothreitol–0.1 mM EDTA in a total volume of 10 μl at room temperature for 1 h. The labeled probe was phenol extracted, ethanol precipitated, and dissolved in 20 μl of 80% (vol/vol) formamide–20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.5)–400 mM NaCl (hybridization buffer). RNA (100 μg) was dissolved in 40 μl of hybridization buffer and incubated with the probe at 44°C for 12 h. Following the addition of 240 μl of S1 nuclease buffer (300 mM NaCl, 18 mM sodium acetate [pH 4.6], 10 mM $ZnSO_4$) and 2 μl of S1 nuclease (384,000 U/μl), the hybridization solution was incubated at room temperature for 30 min. The reaction was terminated by the addition of 10 μl of denatured bakers' yeast tRNA (1 μg/μl), and then phenol extraction and ethanol precipitation of the DNA-RNA hybrids for 1 h at −70°C were carried out. The vacuum-dried samples were dissolved in 10 μl of formamide dye mixture, prepared as described by Bankier and Barrell (5), and heated at 80°C for 5 min before being loaded on either a 4 or 6% (wt/vol) denaturing acrylamide gel (5), which was run at 39 W for 2 to 3 h. A partial *Hin*fI digest of plasmid pUC8, labeled by filling the ends with Klenow polymerase in the presence of dGTP, dCTP, dTTP, and [α-^{32}P]dATP, was used as a molecular size marker (1,613, 913, 517, 396, 140, 75, and 65 nucleotides).

Southern blotting. Samples of genomic DNA (3 to 10 μg) from 17 different species and strains of sulfate-reducing bacteria were digested with *Eco*RI, electrophoresed on a 0.7% (wt/vol) HGT agarose gel as described elsewhere (40), and blotted on Hybond-N hybridization transfer membrane. The DNA was bound to the membrane by UV cross-linking as described above, and the blots were prehybridized for 1 h at 42°C with a solution (pH 7.4) containing 50% (vol/vol) formamide, 1 M NaCl, 0.1% (wt/vol) sodium PP_i, 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) Ficoll 400, 10% (wt/vol) dextran sulfate, and 0.175 mg of denatured salmon sperm DNA per ml. A nick-translated pRub1 probe (20) was then added, and hybridization was continued for 16 h. The Southern blots were washed similarly to the Northern blots, dried, and autoradiographed. The pRub1 probe was removed by washing with 0.5 M NaOH–1.5 M NaCl for 30 min and neutralizing for 30 min with 1 M Tris hydrochloride (pH 8.0)–1.5 M NaCl. Removal of the probe was confirmed by autoradiography, after which the blots were rehybridized with the nick-translated pRbo1 probe.

RESULTS

Nucleotide sequence upstream from *rub*. The nucleotide sequence of the 2.0-kb *Eco*RI fragment, containing the region upstream from the *rub* gene, was determined by the random cloning and dideoxy sequencing procedure of Bankier and Barrell (5), and these data were combined with the sequence of the *rub* gene (37) to obtain a sequence of 2,234 nucleotides (nt) extending from an *Eco*RI to a *Sal*I site (Fig. 1b). An identification of possible coding regions with the aid of the codon probability method of Staden and McLachlan (34) in this 2.2-kb sequence is shown in Fig. 2. Apart from the *rub* gene, this analysis indicates the presence of two other plausible genes in frames a and b. The amino acid sequences derived from these possible genes were compared

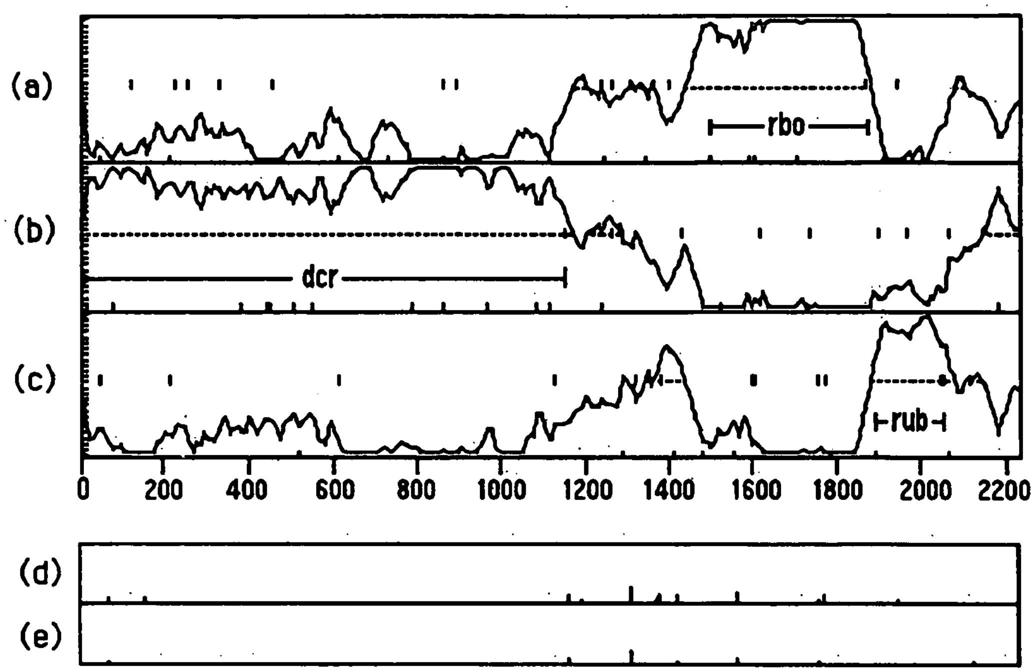

FIG. 2. Statistical analysis of the 2,234-bp region outlined in Fig. 1b. (a to c) The probability that a stretch of sequence (25 bases) is coding is calculated and plotted for each of the three reading frames a, b, and c (36). A codon usage table, which includes all codons of structural genes from *D. vulgaris* Hildenborough, was used as the standard in the calculation. Methionine (ATG) start codons are indicated on the base line, and stop codons are indicated at the half level of each frame. The coding regions for a putative rubredoxin oxidoreductase (*rbo*), a putative *D. vulgaris* chemoreceptor (*dcr*), and rubredoxin (*rub*) are indicated by the calculation as shown in frames a to c, respectively. (d and e) Search for *E. coli* promoters. Sequences with homology to the consensus −35 (TTGACA) and −10 (TATAAT) *E. coli* promoter sequence are indicated in frames d and e, respectively. The scale is in base pairs.

with sequences in the Bionet data base, which indicated unique and extensive homologies with, respectively, desulforedoxin of *D. gigas* (9) and the aspartate and serine chemoreceptors of *Salmonella typhimurium* (30) and *E. coli* (6), respectively. The amino acid sequence that can be derived from the sequenced portion of the *dcr* gene (nt 1 to 1158) lacks approximately 140 NH_2-terminal amino acids, and further nucleic acid sequencing is required for the completion of the sequence of the putative *Desulfovibrio* chemoreceptor protein. The homology of the *dcr* gene product with these two known chemotactically active proteins will therefore not be further discussed here, allowing a focus on the gene immediately preceding the *rub* gene. In view of the homology with desulforedoxin, as discussed below, the upstream gene appears to also encode a redox protein and is tentatively referred to as rubredoxin oxidoreductase, the product of the *rbo* gene.

The nucleotide sequence of the *rub* gene and its upstream region (nt 1301 to 2140) is shown in Fig. 3. Each nucleotide was determined four times on average, at least once on each strand. The gene encoding rubredoxin (nt 1896 to 2057) and the putative *rbo* gene predicted by the codon probability calculations in Fig. 2a (nt 1499 to 1879) have been translated into amino acid sequences. Each gene is preceded by a plausible ribosome-binding site. A search for *E. coli* promoters (Fig. 2d and e) indicates the presence of a promoter consensus sequence (−35, −10) upstream from these two genes, and a potential promoter has been indicated in Fig. 3. A hairpin-loop-forming structure of nine G · C base pairs, which may serve as a transcription terminator, is present immediately downstream from the *rub* gene. Thus, an analysis of the nucleic acid sequence indicates that the two genes may form an operon. Direct experimental evidence for the presence of both genes on a single transcript is presented below.

Northern blotting and S1 nuclease mapping. Northern blots of RNA isolated from both *D. vulgaris* and *E. coli* TG2(pJK29) indicate that a single transcript of approximately 680 nucleotides hybridizes with both the pRbo1 and the pRub1 probes (Fig. 4). The 1.1-kb insert of pJK29 is in the correct orientation for transcription of the *rbo* and *rub* genes from the *E. coli lac* promoter present on the pUC vector. However, a transcript originating from the *lac* promoter and containing both the *rbo* and *rub* genes would be approximately 1,100 nt long, and this size is not consistent with the data in Fig. 4C. The observation of similarly sized transcripts in *D. vulgaris* and *E. coli* indicates that the *D. vulgaris* promoter controlling the transcription of these two genes functions in *E. coli* and may thus resemble the *E. coli* consensus. The observed size of the transcript is sufficiently large to accommodate both the *rbo* and the *rub* genes. Assuming that the transcript terminates at position 2110, on the 3′ side of the hairpin indicated in Fig. 3, the transcriptional start site is placed in the vicinity of position 1430, which is 70 nt upstream from the translational start of the *rbo* gene. The transcriptional start site was next defined more precisely by S1 nuclease mapping. Use of the 913-bp probe gives rise to a major protected fragment of 600 ± 50 nt (average of three experiments), while minor protected fragments of variable, smaller sizes are also observed (Fig. 5). The 600-nt fragment ends at nt 2041 (Fig. 3), indicating a transcriptional start site at nt 1441 ± 50. The presence of a promoter sequence with reasonable homology to the *E. coli* consensus at nt 1355 to 1380 (Fig. 3) suggests a potential start

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                                                                          -35            -10
GCATACTGTGCCTGAAAAGTGAATCACCCCGGACACATCTTTCCGCTCATGGGCTGGACAAGCCCAGATTAGGATTTACTCGTAAAAAATCACGCCGGTCATTGAACAGACCGCCGACAT
         1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420

                                                                                  rbs      M  P  N  Q  Y  E  I  Y  K  C  I  H  C  G
GCTGTACACAGGTATAAACAGGTATTTCCGGCGGCCATTCCCGCCTTTCAGACCACCGACAACGAACATGGAGGCCCCATGCCCAACCAGTACGAAATCTACAAATGCATCCACTGTGGC
         1430      1440      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540

N  I  V  E  V  L  H  A  G  G  G  D  L  V  C  C  G  E  P  M  K  L  M  K  E  G  T  S  D  G  A  K  E  K  H  V  P  V  I  E
AACATCGTCGAAGTCCTGCATGCTGGCGGCGGCGACCTCGTGTGCTGCGGCGAACCCATGAAGCTCATGAAGGAAGGCACTTCTGACGGGGCCAAGGAAAAGCACGTGCCGGTCATCGAG
         1550      1560      1570      1580      1590      1600      1610      1620      1630      1640      1650      1660

K  T  A  N  G  Y  K  V  T  V  G  S  V  A  H  P  M  E  E  K  H  W  I  E  W  I  E  L  V  A  D  G  V  S  Y  K  K  F  L  K
AAGACCGCCAACGGCTACAAGGTCACCGTCGGTTCCGTGGCCCACCCCATGGAAGAGAAGCACTGGATCGAATGGATTGAGCTTGTCGCAGACGGTGTGAGCTACAAGAAGTTCCTGAAG
         1670      1680      1690      1700      1710      1720      1730      1740      1750      1760      1770      1780

P  G  D  A  P  E  A  E  F  C  I  K  A  D  K  V  V  A  R  E  Y  C  N  L  H  G  H  W  K  A  E  A  *       rbs          M  K
CCCGGCGATGCGCCCGAAGCCGAGTTCTGCATCAAGGCCGACAAGGTCGTCGCCCGCGAATACTGCAACCTGCACGGCCACTGGAAGGCCGAAGCCTAACCCCGAGGAATCCACGATGAA
         1790      1800      1810      1820      1830      1840      1850      1860      1870      1880 AvaI 1890      1900

K  Y  V  C  T  V  C  G  Y  E  Y  D  P  A  E  G  D  P  D  N  G  V  K  P  G  T  S  F  D  D  L  P  A  D  W  V  C  P  V  C
AAAGTACGTATGCACCGTCTGCGGTTACGAATACGACCCTGCTGAAGGCGACCCCGACAACGGCGTGAAGCCCGGCACCTCGTTCGACGACCTGCCGGCCGACTGGGTATGCCCCGTGTG
         1910      1920      1930      1940      1950      1960      1970      1980      1990      2000      2010      2020

 G  A  P  K  S  E  F  E  A  A  *  *
CGGCGCCCCCAAGAGCGAATTCGAAGCCGCCTAGTAAGCGCTTTCCATGACGCCGCCGCCGGTTGCCCGTACGGGTTGCCCGCGGCGGCGTTTCTTCGTATAGGGGGGTCATCAGCCCCT
         2030      2040      2050      2060      2070      2080      2090      2100      2110      2120      2130      2140
               EcoRI
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FIG. 3. Nucleotide sequence of the *rbo-rub* operon. The numbering of the sequence is the same as in Fig. 1 and 2. The coding regions of the *rbo* (nt 1499 to 1879) and *rub* (nt 1896 to 2057) genes have been translated with the single-letter amino acid code. Translation stop codons are designated by asterisks. The ribosome-binding sites (rbs) and a plausible promoter (−35, −10) are indicated. A G+C-rich stem-and-loop structure (→←) which could serve as a transcription termination signal is shown.

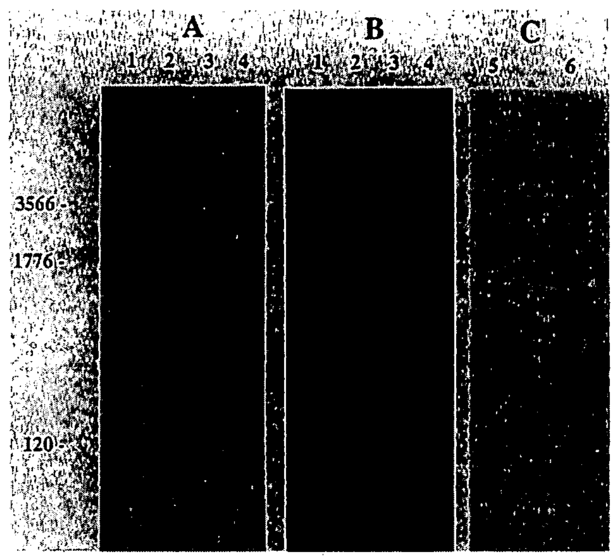

FIG. 4. Northern blotting of total RNA isolated from *D. vulgaris* Hildenborough and *E. coli* TG2(pJK29). (A) Increasing amounts of *D. vulgaris* RNA (25, 50, 75, and 100 μg) were loaded in lanes 1 to 4, respectively. The blot was hybridized to the pRbo1 probe and autoradiographed for 5 days at −70°C. The size markers, 25S rRNA (3,566 bases), 16S rRNA (1,776 bases), and 5S rRNA (120 bases), were visualized by fluorography of the blot following transfer of the ethidium bromide-stained gel. (B) The same blot as in panel A was incubated for 2 min in boiling distilled water to dissociate the pRbo1 probe. Following autoradiography to confirm the dissociation of this probe, the blot was rehybridized to the pRub1 probe. Autoradiography was carried out for 7 days at −70°C. (C) *D. vulgaris* RNA (25 μg) and RNA isolated from *E. coli* TG2 transformed with plasmid pJK29 (100 μg) were loaded in lanes 5 and 6, respectively. Following electrophoresis, the blot was incubated with the pRbo1 probe. Autoradiography was performed for 14 days at −70°C. Use of the pRub1 probe gave identical results (not shown).

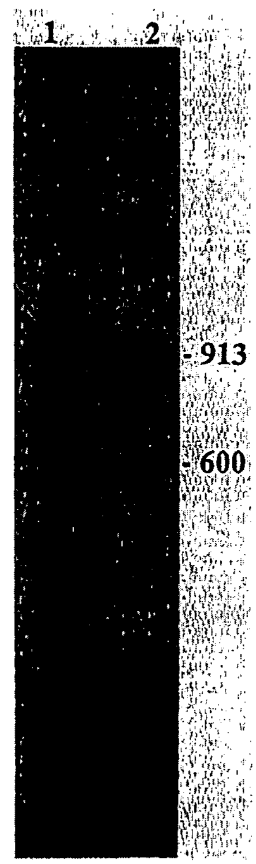

FIG. 5. S1 nuclease mapping of the transcriptional start site of the *rbo-rub* operon. Lanes: 1, 913-nt probe control; 2, *D. vulgaris* RNA (100 μg) hybridized with the 913-nt probe and treated with S1 nuclease; a major protected fragment of 600 nt is indicated. The size of this fragment was derived from the positions of pUC8 × *Hin*fI markers (not shown). Autoradiography was performed for 4 weeks at −70°C.

site in the vicinity of nt 1390, which is within the range predicted by the S1 nuclease mapping results. The smaller (<600-nt) fragments do not map to the same position and are presumed to be artifacts arising from preferential S1 nuclease cleavage (12).

Southern blotting. Southern blots of chromosomal DNA digested with *Eco*RI from 17 different species and strains of sulfate-reducing bacteria, including *D. vulgaris* Hildenborough, were hybridized with the inserts from plasmids pRub1 and pRbo1 as probes. The results obtained are shown in Fig. 6A and B, respectively, as well as in Table 2. These results are discussed in more detail below.

DISCUSSION

Homology of the *rbo* gene product with desulforedoxin. Desulforedoxin is a small redox protein, which has been isolated from *D. gigas* but has so far not been found in other sulfate-reducing bacteria. Its polypeptide chain is only 36 amino acids long and has been determined by protein sequencing (9). As discussed by Le Gall et al. (18), the protein is isolated as a dimer (molecular mass, 7.9 kDa; 3.9 kDa per subunit) with two bound Fe atoms as redox centres and has rubredoxinlike spectroscopic properties. The product of the *rbo* gene (378 nt) is much larger than desulforedoxin (126 amino acids; molecular mass, 14 kDa). A comparison of the two amino acid sequences shows that the homology resides entirely at the NH_2 terminus (Fig. 7). Thus, of the first 36 amino acids of the *rbo* gene product, 19 are identical to desulforedoxin, whereas conservative amino acid changes are frequently found in the nonidentical positions. The four cysteine residues (C-9, C-12, C-28, and C-29), which have been proposed to coordinate to the iron (18), are among the conserved amino acid residues. Another interesting region of homology comprises the three glycine residues (G-22, G-23, G-24), which must represent an important flexible (e.g., loop) region of the molecule. The high degree of homology indicates that at its NH_2 terminus, the *rbo* gene product must have a 4-kDa desulforedoxin domain, which has been fused to a larger polypeptide of 10 kDa. This situation is very reminiscent to that in [Fe] hydrogenase of *D. vulgaris* Hildenborough, which is known to have three iron-sulfur clusters as prosthetic groups coordinated by cysteine residues of the large (46-kDa) subunit. The NH_2 terminus of this large subunit is homologous to bacterial 8Fe-8S ferredoxin (38), indicating that two electron-transferring 4Fe-4S clusters are likely to be present in this part of the molecule in a structure resembling that of ferredoxin (1), while a third unique hydrogen-binding cluster could be coordinated by some of the 10 cysteine residues found in the 40-kDa COOH-terminal part of the molecule. It was proposed, therefore, that the hydrogenase gene originated from a

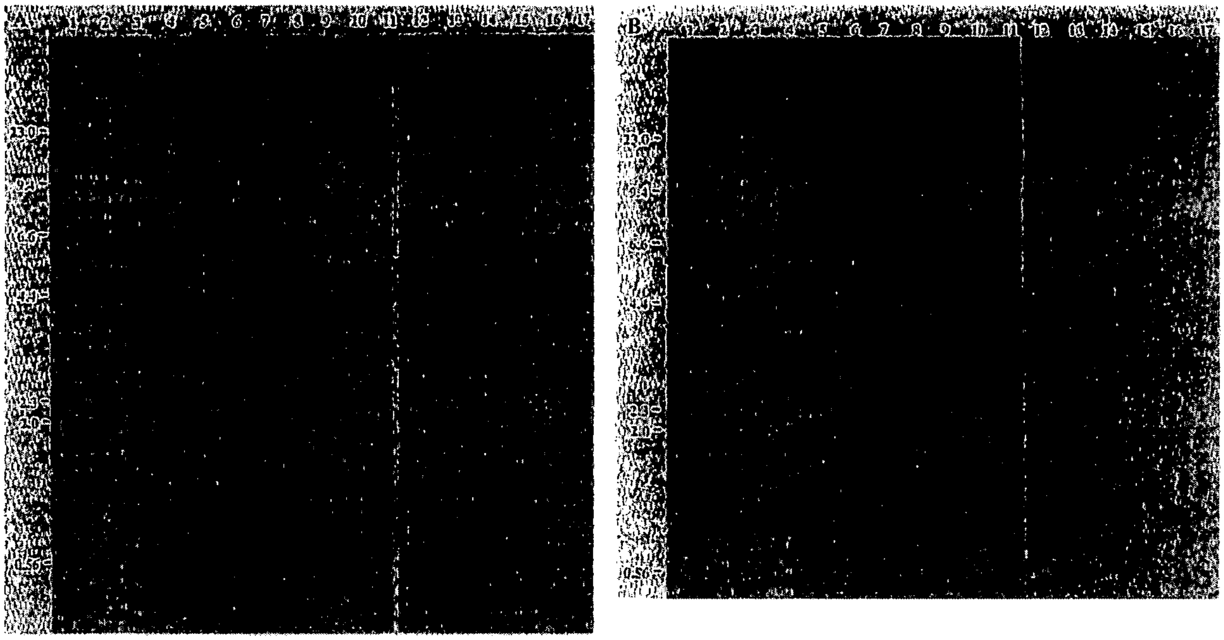

FIG. 6. Southern blotting of *Eco*RI-digested chromosomal DNA (5 to 10 μg) from 17 species and strains of sulfate-reducing bacteria. Lanes: 1, *D. vulgaris* Hildenborough; 2, *D. vulgaris* Wandle; 3, *D. vulgaris* Brockhurst Hill; 4, *D. vulgaris* Groningen; 5, *D. vulgaris* Miyazaki; 6, *D. vulgaris* subsp. *oxamicus* Monticello; 7, *D. desulfuricans* Norway; 8, *D. desulfuricans* Teddington R; 9, *D. desulfuricans* El Agheila Z; 10, *D. desulfuricans* Berre Sol; 11, *D. desulfuricans* Canet 41; 12, *D. gigas*; 13, *D. salexigens* British Guiana; 14, *D. salexigens* California; 15, *D. salexigens*; 16, *D. africanus* Walvis Bay; 17, *D. africanus* Bhengazi. (A) The blots were hybridized with the pRubl probe (containing part of the rubredoxin gene from *D. vulgaris* Hildenborough) and subjected to autoradiography for 8 days at −70°C. The probe was then dissociated from the blots by washing in 0.5 M NaOH–1.5 M NaCl and neutralized by washing with 0.5 M Tris hydrochloride (pH 7.4)–1.5 M NaCl. Complete dissociation of the pRubl probe was confirmed by autoradiography. (B) The blots were then hybridized with the pRbol probe (containing the gene of a putative rubredoxin oxidoreductase from *D. vulgaris* Hildenborough) and subjected to autoradiography for 2.5 days at −70°C. The positions of molecular size markers (bacteriophage λ DNA digested with *Hin*dIII) are indicated in kilobases.

TABLE 2. Hybridization of *Eco*RI-digested chromosomal DNA from 17 different sulfate-reducing bacteria with an *rub*-specific and an *rbo*-specific probe from *D. vulgaris* Hildenborough

Species	Strain	NCIMB no.	Size of hybridizing fragment (kb) for:	
			rub	*rbo*
D. vulgaris subsp. *vulgaris*	Hildenborough	8303	2.0	2.0
	Wandle	8306	2.0	2.0
	Brockhurst Hill	8306	2.0	2.0
	Groningen	11779	15.4	15.4
	Miyazaki		0.5	3.9
D. vulgaris subsp. *oxamicus*	Monticello 2	9442	0.5	1.8
D. desulfuricans subsp. *desulfuricans*	Norway 4	8310		
	Teddington R[a]	8312		7.8
	El Agheila Z	8318		
	Berre Sol	8388	0.5	2.8
	Canet 41	8393	0.5	
D. gigas		9332	1.2	
D. salexigens	British Guiana	8403		
	California 43:63	8364		
		8365		
D. africanus	Walvis Bay	8397	7.1	
	Bhengazi	8401	0.4	3.0

[a] Reclassified as *D. vulgaris* (28).

fusion of genes encoding an electron-transferring ferredoxin and a larger hydrogen-binding polypeptide (38). In the present case, the desulforedoxin domain coincides precisely with the 36 NH_2-terminal amino acids.

Another example of a redox protein that could have arisen by gene fusion is rubrerythrin, which was recently isolated (19) and shown to contain both rubredoxinlike and hemerythrinlike redox centres. The putative *rbo* gene product is distinct from rubrerythrin in its molecular mass (14 versus 22 kDa) and amino acid composition. Also, preliminary protein sequence data on rubrerythrin (D. M. Kurtz, Jr., and J. Le Gall, personal communication) indicate that this protein does not resemble the *rbo* gene product.

Two possible modes of coordination of the two iron atoms by the eight cysteines of a desulforedoxin dimer have been considered by Le Gall et al. (18): the four cysteines of a single subunit could coordinate to the same iron, in which case dimer formation is achieved by noncovalent interactions of the two subunits, or cysteines from both subunits (e.g., C-9, C-12, and C-28 from one and C-29 from the other) could contribute to the coordination of the two irons, causing a partially covalent connection of the two subunits via the two Fe redox centres. In view of its homology with desulforedoxin, the functional form of the *rbo* gene product is also proposed to be a dimer. Only two additional cysteine residues (C-103 and C-115) are present in the remainder of the sequence of the *rbo* gene product (Fig. 7), which is insufficient for the coordination of a third Fe-containing redox centre (e.g., the coordination of a single Fe, a single

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                  5                 10                15
pro-asn-gln-tyr-glu-ile-tyr-lys-cys-ile-his-cys-gly-asn-ile-
ala-asn-glu-gly-asp-val-tyr-lys-cys-glu-leu-cys-gly-gln-val-

                 20                 25                30
val-glu-val-leu-his-ala-gly-gly-gly-asp-leu-val-cys-cys-gly-
val-lys-val-leu-glu-glu-gly-gly-gly-thr-leu-val-cys-cys-gly-

                 35                 40                45
glu-pro-met-lys-leu-met-lys-glu-gly-thr-ser-asp-gly-ala-lys-
glu-asp-met-val-lys-gln

                 50                 55                60
glu-lys-his-val-pro-val-ile-glu-lys-thr-ala-asn-gly-tyr-lys-

                 65                 70                75
val-thr-val-gly-ser-val-ala-his-pro-met-glu-glu-lys-his-trp

                 80                 85                90
ile-glu-trp-ile-glu-leu-val-ala-asp-gly-val-ser-tyr-lys-lys-

                 95                100               105
phe-leu-lys-pro-gly-asp-ala-pro-glu-ala-glu-phe-cys-ile-lys-

                110                115               120
ala-asp-lys-val-val-ala-arg-glu-tyr-cys-asn-leu-his-gly-his-

                125
trp-lys-ala-glu-ala
```

FIG. 7. Comparison of the amino acid sequence of the *rbo* gene product (Fig. 3) with that of desulforedoxin (9). Regions of sequence identity are highlighted by boxes.

2Fe-2S cluster, or a single 4Fe-4S cluster requires four cysteines). However, a third redox centre could be present in the 14-kDa *rbo* gene product, if one assumes that the two subunits can donate both cysteine ligands to the same coordination site. The *rbo* gene product appears quite different from the NADH-rubredoxin oxidoreductase purified from *C. acetobutylicum* which was discussed in the Introduction. The subunit molecular mass is different (41 kDa for the *Clostridium* enzyme), and the search of the Bionet data base did not reveal homologies of the *rbo* gene product with flavin-binding proteins.

In analogy with the [Fe] hydrogenase gene, one could suggest that the *rbo* gene has arisen by fusion of the gene for desulforedoxin with that encoding a 10-kDa redox protein containing a single redox centre. The observation that this gene forms a single operon with the gene encoding rubredoxin could mean that the two proteins are redox partners, but definitive proof for this proposal must await the purification and characterization of this novel redox protein.

Distribution of the *rbo* gene in sulfate-reducing bacteria. The *rub* and *rbo* genes are present on a similarly sized *Eco*RI fragment (Fig. 6) and may thus form an operon as in Fig. 3 in *D. vulgaris* Wandle, Brockhurst Hill, and Groningen. This is no surprise for the first two strains, which are practically identical to the Hildenborough strain (39). However, the Groningen strain is quite distinct from Hildenborough and was shown to lack a gene for [Fe] hydrogenase and to have only a low degree of homology in its gene for cytochrome c_3 (39). Four species of sulfate-reducing bacteria have homologous *rub* and *rbo* genes on different restriction fragments (Table 2; Fig. 6). This could indicate a separation of the two genes in these species or the presence of an additional *Eco*RI site in their *rbo-rub* operon. Finally, a homologous *rub* gene in the absence of a detectable, homologous *rbo* gene is found in three, whereas the converse is found in a single one of the species of sulfate-reducing bacteria examined. *D. gigas*, which has a rubredoxin with 71% sequence identity (37 identical residues in a total of 52) with the protein from *D. vulgaris* Hildenborough (36), the pRub1 probe appears sufficiently homologous for the detection of the *rub* gene on a 1.2-kb *Eco*RI fragment (Fig. 6A, lane 12; Table 2). However, the 53% identity of *D. gigas* desulforedoxin with the N terminus of the *D. vulgaris rbo* gene product (Fig. 7; 19 identical residues in a total of 36) appears insufficient for the detection of the desulforedoxin gene in *D. gigas* (Fig. 6B, lane 12). The data in Fig. 6 should therefore not be interpreted in terms of the presence or absence of a gene encoding a desulforedoxinlike polypeptide in species that fail to hybridize with the pRbo1 probe. They indicate that the *rbo* gene is present in nine other species of sulfate-reducing bacteria and is spatially linked to the *rub* gene in at least four species. The *rbo* gene product could thus function as a redox protein in some but not all species of sulfate-reducing bacteria.

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